

Antimicrobial activity of coffee-based solutions and their effects on *Streptococcus mutans* adherence

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Abstract

The aim of this study was to evaluate the antimicrobial activity of different coffee solutions and their effects on the adherence of *Streptococcus mutans* to glass surface. Coffee solutions were prepared with three commercial products (Pilão, Mellita and Café do Ponto) by two different methods (simple and boiled) (n=15). A control group was also included in the study. For antimicrobial activity testing, tubes containing coffee solution and culture medium were inoculated with a suspension of *S. mutans* ATCC 35688 and incubated for 1min, 1h, 2h and 4h. Serial dilutions and plating on BHI agar were performed. *S. mutans* adherence to glass in presence of the different coffee solutions was also tested. The number of adhered bacteria (CFU/mL) was determined by plating method. The results were statistically analyzed by ANOVA and Tukey's test. The tested coffee solutions did not reduce the number of colony forming units of *S. mutans* in relation to the control at all evaluation periods. All the solutions reduced significantly the adherence of *S. mutans* to the glass surface in relation to control. The tested coffee solutions did not present any antimicrobial effect on *Streptococcus mutans*, however, all the coffee solutions reduced significantly the adherence of *S. mutans* to the glass surface.

Key Words:

Streptococcus mutans, *Coffea arabica*

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Introduction

Caries are still considered one of the main problems of public health¹ and many researchers in the world have been searching for alternatives to prevent the occurrence of this process. Mutans streptococci are the main etiologic agent of caries, especially in smooth surfaces².

Several previous studies²⁻⁸ demonstrated the activity of natural extracts (green, black and oolong teas, cacao, propolis) on the dental biofilm and caries development. The capacity of some extracts to affect the synthesis of extracellular polysaccharides may have an important role in the determination of their anti-cariogenic potential⁹⁻¹⁰.

Antimicrobial and anticariogenic properties of tea have been extensively studied. Several types of tea present in their composition substances with anticariogenic potential such as caffeine^{3-4,11-12}, theobromine and xanthine³. Tannic acid, found in several types of tea, is cited as an important inhibitor of bacterial growth and glucosyltransferase activity¹¹⁻¹⁵. This acid is also able to form stable complexes with proteins rich in proline that are present in saliva and are directly involved in the adherence of oral bacteria to the acquired pellicle¹⁵. Previous studies^{3-4,7,11} on mate, black, green and oolong teas showed that these infusions are related to the inhibition of bacterial growth and adherence to dental surface, and reduction in the production of acid and extracellular polysaccharides. Studies on animals showed that the inclusion of tea in the diet reduced significantly the incidence of dental caries^{3,12,15}.

Coffee is classified in the *Rubiaceae* family, *Coffea* genus, and the species cultivated in Brazil are *Coffea arabica* and *Coffea canephora*, known as "arabic coffee" and "robust coffee", respectively. Arabic coffee represents more than 75% of the world-wide trade¹⁶. Coffee grain is composed by water, mineral substances, glucides, lipids, organic acids, alkaloids, tannic acids, theobromine, caffeine and several vitamins¹⁷.

Few studies on the antimicrobial activity of coffee-based solutions are found in the literature. Toda et al.¹⁶ related the effects of coffee on microbial species such as *Staphylococcus aureus*, *Salmonella thypi*, *Shigella dysenteriae*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Yersinia enterocolitica* and attributed this bactericide effect to the tannic acid. *In vitro* studies showed that extracts of coffee may inhibit glucosyltransferase in several oral streptococci¹³⁻¹⁴. Landucci et al.¹⁸ and Daglia et al.¹⁹ related the anti-adhesive effect of coffee on the adherence of *Streptococcus mutans*. The aim of this study was to evaluate the antimicrobial activity of coffee-based solutions from different origins and obtained by two distinct methods on *S. mutans* and their effects on the adherence to glass surface.

Material and Methods

Three extensively available commercial coffee trade-marks (Café do Ponto, São José dos Campos-SP, Brazil; Pilão,

Barueri-SP, Brazil; and Mellita, Avaré-SP, Brazil) were included in the study. The proportion of 20 g of coffee powder to 250 mL of fluoride water (1 ppm) was adopted as the standard solution²⁰.

Coffee solutions were obtained by two different methods: a) Simple: boiling water was dropped through the powder coffee in a paper filter (Mellita, Avaré-SP) and; b) Boiled: coffee powder was boiled with the water for 2 min and then filtered through the paper filter (Mellita, Avaré-SP, Brazil).

Antimicrobial activity testing

Nine milliliters of each coffee solution obtained were put into 15 tubes containing dehydrated culture medium (brain heart infusion broth, BHI – Difco, Detroit, USA and 10% sucrose) and immediately submitted to autoclave sterilization. Growth control consisted in tubes containing BHI broth supplemented with sucrose 10% and fluoride distilled water (1 ppm). The seven experimental groups included in the study are presented in Table 1.

Table 1 – Experimental groups (n=15) included in the study according to the trade-marks and preparation of the coffee infusions

Groups	Trade-mark	Preparation
G1	Pilão	Simple
G2	Pilão	Boiled
G3	Mellita	Simple
G4	Mellita	Boiled
G5	Grains	Simple
G6	Grains	Boiled
G7 (Control)	Water	-

A standardized *Streptococcus mutans* ATCC 35688 suspension containing 1×10^6 cells/mL was obtained by spectrophotometry. After sterilization, each tube was inoculated with 1 mL of the bacterial suspension and incubated at 37°C in atmosphere of 5% CO₂ for 1 min, 1 h, 2 h and 4 h. After the incubation period, 0.1 mL of each tube's content was plated in duplicate on BHI agar. Plates were incubated and the logarithm of colony forming units per milliliter (log UFC/mL) was obtained for each group.

Streptococcus mutans adherence to glass

Nine milliliters of each the coffee solution were distributed into 15 tubes containing dehydrated culture medium (BHI broth + 10% sucrose). Then, standardized glass specimens (diameter= 2 mm, length= 5 cm) were added to these tubes and submitted to autoclave sterilization. For evaluation of bacterial adherence evaluation, one milliliter of standardized *S. mutans* suspension (1×10^6 cells/mL) obtained by spectrophotometry was inoculated into each tube and then

incubated for 90 minutes at 37°C in atmosphere of 5%CO₂ (Otake et al.¹⁵). After the period of incubation, the specimens were transferred to tubes containing buffered phosphate saline pH 7.2 (PBS, Sigma, St. Louis, USA) and glass beads. Tubes were submitted to agitation (Phoenix AP56, São Paulo, Brazil) and from this initial suspension, dilutions of 10⁻¹ and 10⁻² were obtained in sterilized NaCl 0.85% saline solution (Labsynth, Diadema, SP, Brazil). Then, aliquots of 0.1 mL of each dilution were plated in duplicate on BHI agar and incubated for 48 h at 37°C in atmosphere of 5% CO₂. After this period, the number of colonies was counted and the value of logarithm of colony forming units per milliliter was calculated (log CFU/mL).

The results obtained were analyzed statistically by ANOVA and Tukey's test (5%).

Results

Antimicrobial activity testing

Mean, standard deviation and median values of CFU/mL obtained for the experimental groups at each period of evaluation are represented in Table 2.

No statistically significant difference was observed among log CFU/mL values obtained for experimental groups and control (p>0.05).

Table 2 – Mean and standard deviation values (log CFU/mL) obtained for the experimental groups after 1 min, 1 h, 2 h and 4 h contact of *Streptococcus mutans* and coffee solutions

Groups	Mean ± standard deviation			
G1	5.80 ± 0.13	5.88 ± 0.10	5.59 ± 0.14	5.62 ± 0.26
G2	5.65 ± 0.16	5.65 ± 0.15	5.66 ± 0.08	5.69 ± 0.06
G3	5.50 ± 0.10	5.51 ± 0.19	5.50 ± 0.17	5.85 ± 0.13
G4	5.54 ± 0.14	5.67 ± 0.17	5.50 ± 0.17	5.61 ± 0.12
G5	5.84 ± 0.12	5.81 ± 0.08	5.84 ± 0.10	5.76 ± 0.13
G6	5.37 ± 0.09	5.56 ± 0.07	5.44 ± 0.18	5.48 ± 0.18
G7	5.55 ± 0.14	5.65 ± 0.17	5.58 ± 0.14	5.65 ± 0.14

G1: Simple Pilão; G2: Boiled Pilão; G3: Simple Mellita; G4: Boiled Mellita; G5: Simple Grains; G6: Boiled Grains; G7: Control.

S. mutans adherence to glass

The results obtained for the analysis of *S. mutans* adherence to glass in the presence of the coffee solutions are presented in Table 3. ANOVA and Tukey's test results showed significantly higher counts for group 7 (control) (p=0.00). Groups 1 (Pilão, simple), G2 (Pilão, boiled), G3 (Mellita, simple) and G4 (Mellita, boiled) presented similar values of CFU/mL (p=0.99). The lowest counts were obtained for groups 5 (Café do Ponto, simple) and 6 (Café do Ponto, boiled) (p=0.00).

Table 3 – Mean, standard deviation and median values (log UFC/mL) obtained in the analysis of the adherence to glass

Groups	Mean ± Standard deviation	Median	Homogenous groups*
G1	2.77 ± 0.17	2.80	A
G2	2.82 ± 0.24	2.88	A
G3	2.81 ± 0.21	2.84	A
G4	2.75 ± 0.26	2.79	A
G5	2.40 ± 0.31	2.49	B
G6	2.50 ± 0.27	2.49	B
G7	3.18 ± 0.12	3.17	C

G1: Simple Pilão; G2: Boiled Pilão; G3: Simple Mellita; G4: Boiled Mellita; G5: Simple Grains; G6: Boiled Grains; G7: Control.

* Different letters indicate statistically different values.

Discussion

Previous studies have been performed to evaluate the effect of several natural extracts on dental biofilm and caries development²⁻⁸. Studies on the antimicrobial and anticariogenic properties of coffee species are not commonly found in the literature even though it is considered the best-known and one of the most appreciated drink in the world²¹. In our country, besides the historical aspect, coffee represents one of the most consumed products by the population¹⁸. Several commercial marks of coffee are available in Brazilian market and for this study, three extensively available trade-marks of coffee were selected.

Although coffee and tea share the presence of some components, in this study no antimicrobial activity of coffee on *S. mutans* could be evidenced. No differences were observed among the final counts of experimental groups and control, indicating that the coffee solutions tested were not able to reduce significantly the number of *S. mutans* viable cells at all the periods of contact. Similar results were obtained previously in studies on different types of tea^{1,22-25}. The antimicrobial activity of coffee against other bacterial species was previously related in the literature¹⁶. No previous study evaluating the antimicrobial potential of coffee on *S. mutans* was found in the literature.

Although no antimicrobial effect was observed, the tested coffee solutions reduced significantly the adherence of *S. mutans* to glass surface. These results suggest potential anticariogenic activity considering that adhesion of *S. mutans* is considered an essential step in the initiation and development of dental caries¹⁸⁻¹⁹. This effect may probably be associated with the capacity to affect the synthesis of extracellular polysaccharides^{12,14}. Also, previous *in vitro* studies showed that coffee may inhibit glucosyltransferase in several oral streptococci¹³⁻¹⁴. Landucci et al.¹⁸ and Daglia et al.¹⁹ also related the anti-adhesive effect of coffee on the adherence of *Streptococcus mutans*. Similar studies on the adherence on dental enamel and dentine could generate

interesting data on this subject.

Several authors related the anticariogenic activity to tannic acid compound^{2,12,25} that is also present in coffee. Considering that coffee is formed by several substances such as water, mineral substances, glucides, lipids, organic acids, alkaloids, tannic acids, theobromine, caffeine and several vitamins¹⁷, the isolated evaluation of each compound may clarify the specific agent related to its anti-adhesive effect.

Although antimicrobial activity was expected based on the presence of some common components with tea, that exhibit this property, it was not observed in this study. On the other hand, the coffee-based solutions tested presented possible anti-cariogenic activity related to the reduction of *S. mutans* adherence. These data are very promising considering that adherence is one of the main virulence factor of this species. Other studies including more types of coffee, isolated compounds of coffee, anti-adhesive activity on dental enamel and dentine and also *in vivo* studies are essentially necessary to highlight the clinical applications of these findings.

Considering the results obtained in this study, it can be concluded that the different coffee solutions did not present any antimicrobial effect on *Streptococcus mutans* ATCC 35688. All the studied coffee solutions reduced significantly the adherence of *S. mutans* to the glass surface.

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