Abstract

Aim: Coffee extract has demonstrated significant antimicrobial properties against various Gram-positive and Gram-negative bacteria. 0.2% chlorhexidine, a potent allopathic reagent, in the mouthwash form is considered the gold standard of chemical plaque control. The aim of this study was to evaluate the antimicrobial efficacy of different concentrations of coffee extract with 0.2% chlorhexidine mouthwash on the following Gram-negative periodontal pathogens: *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans* under *in vitro* conditions. **Materials and Methods:** Bacterial suspensions of *P. gingivalis*, *P. intermedia*, *F. nucleatum* and *A. actinomycetemcomitans* were inoculated in agar plates with four, 5 mm diameter wells. Various concentrations of coffee extract and chlorhexidine mouthwash were added into wells in different plates and then incubated at 37°C for 48 h. The diameter of zones of inhibition was measured, and statistical analysis was done. **Results:** 0.2% chlorhexidine mouthwash showed greatest zone of inhibition against all periodontal pathogens. Coffee at a concentration of 20% and 15% showed activity against *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans*. *F. nucleatum* was resistant to all concentrations of coffee extract. **Conclusion:** Coffee extract possesses antimicrobial activity against the various periodontal pathogens though not as efficacious as the standard chlorhexidine.

**Keywords:** *Aggregatibacter actinomycetemcomitans*, chlorhexidine, *Porphyromonas gingivalis*

Introduction

Periodontal diseases are among the most widespread oral bacterial diseases of humanity that affect 15–20% of the world’s population, including Asia, eventually leading to tooth loss, if left untreated.[1,2] Although bacteria belonging to more than 630 different taxa exist in the oral cavity, only 10–15 bacterial species are recognised as potential periodontal pathogens.[3] Of them, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* are recognised as the major pathogens for initiation and progression of destruction of tooth supporting structures. The levels of *P. gingivalis*, *P. intermedia* and other anaerobic bacteria are seen to increase in adult onset periodontitis. While *P. gingivalis* and *A. actinomycetemcomitans* are strongly associated with localised aggressive periodontitis, *P. intermedia* is predominantly associated with the development of necrotising ulcerative gingivitis.[4] Longitudinal and retrospective studies have demonstrated an increased risk of periodontal breakdown in *A. actinomycetemcomitans* and *P. gingivalis* positive sites and better post-treatment results in their absence.[5]
Coffee is one of the most widely consumed beverages in the world. Hence, understanding its composition and actions on the human body are of scientific benefit. Coffee bean extract has been known to have antimicrobial effects against both Gram-positive and Gram-negative bacteria as far back as 1989. Some components in coffee such as caffeine, volatile and non-volatile organic acids, phenols and aromatic compounds are reported to have antimicrobial activity. Chlorogenic acid (CGA) and caffeic acid, which are non-volatile organic acids found in coffee, inhibit the growth of some Gram-positive microorganisms such as *Staphylococcus aureus*, *Bacillus cereus*, Lactobacillus bulgaricus, Streptococcus lactis, Streptococcus faecalis and Gram-negative bacteria such as *Escherichia coli*, Salmonella typhi and *Pseudomonas aeruginosa*.[7]

Coffee has demonstrated significant antibacterial properties against the cariogenic bacteria *Streptococcus mutans* and *Streptococcus mitis* and has also been found to be effective against the periodontal pathogens *P. gingivalis* and *P. intermedia*, as well as *Candida albicans*. Most laboratory preparations of coffee and its extract are not similar to the coffee preparations used commercially or at home. Many coffee producers often blend up to 30% of chicory with coffee, which cuts down on the caffeine content and may have other health benefits.[9-13]

To the best of our knowledge, there are currently no studies assessing the efficacy of commercially available coffee extract with the various periodontal pathogens, using the disc diffusion method. Hence, the present study will comprehensively report the antimicrobial potential of coffee on these key periodontal pathogens. It will also assess and compare the *in vitro* efficacy of coffee extract with the gold standard 0.2% chlorhexidine.

**Materials and Methods**

The study was designed as an *in vitro* study. Before conducting the study, necessary ethical approval was obtained from the Institutional Ethics Committee. Other related approvals from the concerned authorities were obtained for the necessary microbial analysis. Materials used in the study were 20%, 15%, 10% and 5% coffee extract, chlorhexidine mouthwash 0.2% as a positive control and distilled water as a negative control. Microorganisms included were *P. gingivalis*, *P. intermedia*, *F. nucleatum* and *A. actinomycetemcomitans* strains. Test materials included blood agar plates, Petri-dishes and a digital Vernier caliper. A total of 120 samples were taken. Samples were divided into 6 reagent groups, as mentioned above ×4 strains of microorganisms ×5 repetitions. e.g. 20% coffee extract was tested on *P. gingivalis* 5 times.

**Preparation of extract**

Powdered coffee was obtained commercially. Its constituent ingredients were 80% coffee canephora and 20% chicory, and this blend was roasted to a medium degree. Aqueous extracts of coffee were obtained by a coffee brewing procedure based on a previous study by Antonio et al.[9] Preparation of 20% extract was done by percolating 100 ml of pre-boiling (95°C) sterile water through 20 g of ground coffee. A filter paper was used to filter the extracts. After preparation of 20% aqueous extract of coffee, further dilution was done using sterile water to obtain the concentrations of 15%, 10% and 5%.

**Disc diffusion method**

The antibacterial activity of coffee extract and 0.2% chlorhexidine was determined by disc diffusion method. Culture medium used was blood agar. A loop or swab method was used to transfer microbial colonies to the agar plates. Turbidity was visually adjusted with the broth to equal that of a 0.5 McFarland turbidity standard that had been vortexed. Alternatively, the suspension was standardised with a photometric device. Within 15 min of adjusting the inoculums to a McFarland 0.5 turbidity standard, a sterile cotton swab was dipped into the inoculum and rotated against the tube wall above the liquid to remove excess inoculum. Then, swabbing the entire surface of the agar plate was done thrice, rotating plates approximately 60º to ensure even distribution. Care was taken to avoid extra hitting of the sides of the plates to prevent creating aerosols.

Inoculated plates were allowed to stand for at least 3 min, but no longer than 15 min before making wells. A hollow tube of 5 mm diameter was heated and pressed above the inoculated agar plates and was removed immediately, making a well in the plate. Likewise, four wells of 5 mm diameters each were made on each plate. Different concentrations of coffee extract, 0.2% chlorhexidine mouthwash and distilled water were added in the wells assigning each plate for each of the solution. Within 15 min of compound application, plates were shifted to an anaerobic jar, which was kept in an incubator for 48 h. After incubation was complete, plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of zones of inhibition was measured for all the wells using a digital Vernier caliper. The mean score of zones of inhibition was calculated for each solution, respectively.

**Results**

0.2% chlorhexidine mouthwash showed the greatest zone of inhibition against all periodontal pathogens. Coffee at a concentration of 20% and 15% showed antimicrobial activity against *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans*. *F. nucleatum* was resistant to all concentrations of coffee extract [Table 1 and Figures 1-3].

**Discussion**

Available antiplaque agents are based on the use of broad-spectrum antimicrobial agents such as chlorhexidine, quaternary ammonium compounds and antibiotics. These synthetic antimicrobial agents have resulted in considerable side effects, antimicrobial resistance and the emergence of previously uncommon infections owing to their improper usage.[10] Among all the natural products with antibacterial
properties, coffee is the most popular owing to its safety, pleasant odour and taste.

Previous studies have been performed to evaluate the antimicrobial effect of several different types of coffee extracts in vitro,\[^{8,9,12-15}\] on dental biofilm\[^{9,11,15}\] and on caries development.\[^{9,13}\]

Among the scientific literature available, there is sufficient evidence that the nature of the coffee extract may influence its antimicrobial efficacy. Factors that are considered to have a significant effect include species of the coffee beans, degree of roasting of coffee beans, decaffeination, composition and blend of coffee and last but not the least, the concentration of the extract/solution. Of the two widely used species of coffee, i.e. Arabica and Canephora/Robusta, the latter has been shown to have better antimicrobial efficacy, at least against the cariogenic bacteria \textit{S. mutans}.\[^{14}\] Green or non-roasted coffee appears to have the highest antimicrobial activity. Furthermore, degree of roasting is inversely proportional to its antimicrobial efficacy.\[^{14}\] This is attributed to the decomposition of CGAs, the main active compound in coffee, during roasting.\[^{7}\] Decaffeination also lowers the efficacy of coffee as an antibacterial agent.\[^{14}\]

Few studies have been conducted on different compositions of coffee powder. In a study by Sharma \textit{et al.},\[^{13}\] they have used 100% pure coffee, 100% pure chicory and various combinations of coffee and chicory blends. They noted that chicory exerted greater antimicrobial action compared to coffee, and that coffee showed greater reduction in the adherence of \textit{S. mutans} to glass surface. Both these effects were observed in a dose-dependant manner. However, these results were restricted to their effects on the cariogenic bacteria \textit{S. mutans} and cannot be readily extrapolated to the various periodontal pathogens.

Various concentrations of coffee extract have been tested in several studies. One of the commonly used formulations is that described by Antonio \textit{et al.},\[^{11}\] to achieve a 20% stock solution (20 g/100 ml) by percolating 100 ml of distilled water through 20 g of coffee powder and filtering the extracts. Other concentrations that have been used range from 100 μg/ml to 0.2% chlorhexidine mouthwash.

| Table 1: Mean values of zone of inhibition |
|------------------------------------------|
| PG (mm)| PI (mm)| FN (mm)| AA (mm)|
| 20% coffee extract | 12 | 12 | R* | 12 |
| 15% coffee extract | 11 | 11 | R* | 10 |
| 10% coffee extract | 10 | R* | R* | R* |
| 5% coffee extract | R* | R* | R* | R* |
| 0.2% chlorhexidine mouthwash | 18 | 16 | 17 | 17 |
| Distilled water | R* | R* | R* | R* |

*R: Resistant. PG: \textit{Porphyromonas gingivalis}, PI: \textit{Prevotella intermedia}, FN: \textit{Fusobacterium nucleatum}, AA: \textit{Aggregatibacter actinomycetemcomitans}
down to 0.2 μg/ml.[12] The latter study has also shown that coffee at very low concentrations of 0.2 μg/ml shows antibacterial properties against *P. gingivalis, P. intermedia* and *A. actinomycetemcomitans* but shows activity against *F. nucleatum* only at a higher concentration of 3.125 μg/ml. Overall, it would appear that an increase in the concentration of coffee extract significantly increases the antibacterial activity of coffee.

The current study has used a commercially available ground and medium roasted blend of coffee canephora and chicory in the ratio 80:20, respectively. The results have shown that this type of coffee also possesses antimicrobial properties against the periodontal pathogens, *P. gingivalis, P. intermedia* and *A. actinomycetemcomitans*, though not as robust as that of the gold standard 0.2% chlorhexidine. This can be anticipated as other studies showing equal efficacy[8] of 20% coffee extract to 0.2% chlorhexidine have used pure green coffee extracts without roasting and measures to ensure highest antimicrobial efficacy. This study also showed a resistance of *F. nucleatum* to all coffee extracts which is in accordance with another study,[12,15] in which it was noted that minimum inhibitory concentration (MIC) of *F. nucleatum* to coffee extract was 16 times higher than that of *P. gingivalis, P. intermedia* and *A. actinomycetemcomitans*.

Perhaps the most convincing evidence to date on the clinical effectiveness of coffee on periodontal disease comes from a longitudinal study by Ng *et al.*,[16] in which they observed a small, but statistically significant reduction in the number of teeth with periodontal bone loss. Moreover, it has also been concluded that though the benefits of coffee towards the periodontium maybe currently unclear, another important finding is that its consumption is in no way harmful towards periodontal health.[16,17] Coffee consumption has also been explored widely in the medical field and various studies have observed benefits such as reduced risk for mortality,[18] type II diabetes,[19] stroke in women[20] and cancer.[21]

Therefore, based on the findings of this study, we believe that further clinical trials of short- and long-term durations hold promise for assessing the clinical efficacy of coffee in the field of periodontics. There are, however, a few drawbacks to the present study. The stock solution of 20% was used based on a study by Antonio *et al.*[11] but could have been modified or increased to see if increased concentrations would increase the antibacterial activity or it would achieve a ceiling effect. The disc diffusion method used in this study is capable of measuring varying degrees of antibacterial activity; however, it is not possible to deduce MIC or minimum bactericidal concentration. Hence, the ideal concentration of coffee extract still remains unknown. Further analysis of the concentration of coffee in each cup would be required to assess if the results of this study or any other can be applied to an external population. Another welcome addition to the study would be the study of the efficacy of coffee extract on *ex vivo* or *in vivo* periodontal biofilms.

**Conclusion**

Based on our present findings, we can conclude that commercially available coffee extract does indeed possess antimicrobial activity against the periodontal pathogens *P. gingivalis, P. intermedia* and *A. actinomycetemcomitans*. However, its ideal concentration and clinical efficacy need to be addressed in future clinical trials of short and long duration, along with its action on the periodontal biofilm.

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**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Corbet EF. Periodontal diseases in Asians. J Int Acad Periodontol 2006;8:136-44.
2. Corbet EF, Leung WK. Epidemiology of periodontitis in the Asia and Oceania regions. Periodontol 2000 2011;56:25-64.
3. Emami S, Gunjigam GV, Mehta DS. Determination of the antibacterial activity of simvastatin against periodontal pathogens, *Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans*: An *in vitro* study. Contemp Clin Dent 2014;5:377-82.
4. Carranza FA. In: Carranza’s Clinical Periodontology. 9th ed. Philadelphia, USA: W.B. Saunders Company; 2002.
5. Baehni P, Tsai CC, McArthur WP, Hammond BF, Taichman NS. Interaction of inflammatory cells and oral microorganisms. VIII. Detection of leukotoxic activity of a plaque-derived Gram-negative microorganism. Infect Immun 1979;24:233-43.
6. Toda M, Okubo S, Miyoshi R, Shimamura T. The bactericidal activity of tea and coffee. Lett Appl Microbiol 1989;8:123-5.
7. Fardiaz S. Antimicrobial activity of coffee (Coffea robusta) extract. ASEAN Food J 1995;10:103-6.
8. Mehta VV, Rajesh G, Rao A, Shenoy R, Pai M. Antimicrobial efficacy of *Panica granatum* mesocarp, *Nelumbo nucifera* leaf, *Psidium guajava* leaf and *Coffea canephora* extract on common oral pathogens: An *in vitro* study. J Clin Diagn Res 2014;8:ZC65-8.
9. Antonio AG, Iorio NL, Pierro VS, Candreva MS, Farah A, dos Santos KR, et al. Inhibitory properties of *Coffea canephora* extract against oral bacteria and its effect on demineralization of deciduous teeth. Arch Oral Biol 2011;56:556-64.
10. Li M, Xu Z. Quercetin in a lotus leaves extract may be responsible for antibacterial activity. Arch Pharm Res 2008;31:640-4.
11. Antonio AG, Iorio NL, Farah A, Netto dos Santos KR, Maia LC. Effect of *Coffea canephora* aqueous extract on microbial counts in *ex vivo* oral biofilms: A case study. Planta Med 2012;78:755-60.
12. Bharath N, Sowmya NK, Mehta DS. Determination of antibacterial activity of green coffee bean extract on periodontogenic bacteria like *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum and Aggregatibacter actinomycetemcomitans*: An *in vitro* study. Contemp Clin Dent 2015;6:166-9.
13. Sharma R, Reddy VK, Prashant G, Ojha V, Kumar NP. Antimicrobial and anti-adherence activity of various combinations of coffee-chicory solutions on *Streptococcus mutans*: An *in vitro* study. J Oral Maxillofac Pathol 2014;18:201-6.
14. Antonio AG, Moraes RS, Perrone D, Maia LC, Santos KR, Iório NL, *et al.* Species, roasting degree and decaffeination influence the antibacterial activity of coffee against *Streptococcus mutans*. Food Chem 2010;118:782-8.
15. Meckelburg N, Pinto KC, Farah A, Iório NL, Pierro VS, dos Santos KR, *et al.* Antibacterial effect of coffe: Calcium concentration in a culture containing teeth/biofilm exposed to *Coffea Canephora* aqueous extract. Lett Appl Microbiol 2014;59:342-7.
16. Ng N, Kaye EK, Garcia RI. Coffee consumption and periodontal disease
in males. J Periodontol 2014;85:1042-9.
17. Duarte PM, Reis AF. Coffee consumption has no deleterious effects on periodontal health but its benefits are uncertain. J Evid Based Dent Pract 2015;15:77-9.
18. Freedman ND, Park Y, Ahnet CC, Hollenbeck AR, Sinha R. Association of coffee drinking with total and cause-specific mortality. N Engl J Med 2012;366:1891-904.
19. Ding M, Bhupathiraju SN, Chen M, van Dam RM, Hu FB. Caffeinated and decaffeinated coffee consumption and risk of type 2 diabetes: A systematic review and a dose-response meta-analysis. Diabetes Care 2014;37:569-86.
20. Lopez-Garcia E, Rodriguez-Artalejo F, Rexrode KM, Logroscino G, Hu FB, van Dam RM. Coffee consumption and risk of stroke in women. Circulation 2009;119:1116-23.
21. Yu X, Bao Z, Zou J, Dong J. Coffee consumption and risk of cancers: A meta-analysis of cohort studies. BMC Cancer 2011;11:96.