Original Article

Coffee pulp: From a by-product of coffee production to a potential anticariogenic mouth rinse! An *in vivo* study

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Abstract

Background and Objectives: Dental caries is a prevalent disease despite various efforts made toward its prevention. The drawbacks of the available preventive agents have led to the quest for a potentially more effective agent with fewer adverse effects. Coffee, a local produce of Coorg, is one such herbal alternative. This study aims to assess the potential antimicrobial activity of Robusta coffee pulp extracts on *Streptococcus mutans*.

Methodology: A total of 39 participants were divided into three groups with 13 participants each, after obtaining ethical clearance and informed consent: Group A (negative control), sterile water; Group B (positive control), 0.2% chlorhexidine mouth rinse; and Group C, 2.5% coffee pulp extract rinse (prepared according to minimum inhibitory concentration). The saliva samples were collected from the patients in a sterile Eppendorf tube at prerinse for baseline, at 1-h postrinse, and at the end of 2 weeks. The *S. mutans* colony count was done using image-based software analysis. The acquired data were statistically analyzed with one-way ANOVA and repeated measures ANOVA followed by *post hoc* Tukey's test.

Results: Coffee pulp mouth rinse ($P = 0.035$) and positive control ($P = 0.036$) groups showed a statistically significant reduction in the microbial count at 2 weeks postrinse (compared to the negative control group).

Conclusion and Clinical Relevance: The coffee pulp extract-based mouth rinse is a potential anticariogenic agent that offers few advantages over chlorhexidine as no instances of staining, altered taste, or any allergic reactions were reported by the subjects.

Keywords: Caries prevention; Robusta coffee extracts; *Streptococcus mutans*

INTRODUCTION

Dental caries is estimated to have a prevalence of 2.03 billion in the global burden of disease.[1] With the progress of Minimal Invasive Dentistry, there has been an increased emphasis on caries prevention.[2,3] Mouth rinsing was established as a mass prophylactic method for the prevention of dental caries in children and adolescents in the 1960s and has shown average efficacy of caries reduction of between 20% and 50%.[4] A variety of synthetic antimicrobial mouthwashes are available which have been shown to inhibit plaque formation, reduce gingival inflammation, and also prevent dental caries. Despite reducing plaque, chlorhexidine (the most commonly used mouth rinse) does not concurrently reduce caries as concluded by a Cochrane review which considered eight clinical trials in adolescents and children.[5] Chlorhexidine has also been reported to cause staining and transient impairment of taste perception.[6] Studies on the effect of chlorhexidine on the bond strength of dental adhesive systems on dentin have reported conflicting results.[7]

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Although fluoride mouth rinses have generally proved to be effective in controlling caries in clinical studies, their benefits in adults have been less well documented.[8] Furthermore, when the dosage and use are not monitored carefully, it can have toxic effects.[9]

Herbal agents due to their accessibility, low cost, and effectiveness have the potential to become one of the most effective ways of preventing dental caries.[10] Coffee is one such herbal alternative. It is one of the most widely consumed beverages in the world and a local product of Coorg in Karnataka. It is a pharmacologically active tropical plant prompting numerous studies on its potential actions. Nevertheless, the study of the antibacterial activity of the various by-products of coffee is seemingly limited. Coffee pulp is known to have antibacterial properties,[11] but their action against *Streptococcus mutans* is yet to be evaluated. This study aims to assess the potential antimicrobial activity of Robusta coffee pulp extracts on *S. mutans*.

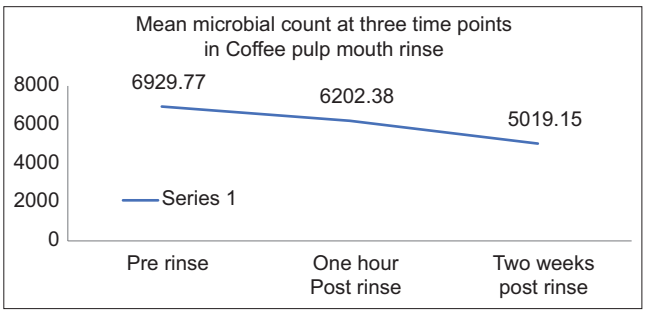
METHODOLOGY

In a previous study, the minimum inhibitory concentration of the extract was determined to be 12.5 mg/ml after serial dilutions and microplating;[12] therefore, the mouthwash was prepared at a concentration of 25 mg/ml which is 2.5% concentration with 5% dimethyl sulfoxide (to improve the solubility of the extract in distilled water).

Sample size estimation was done using the formula: $n = (Z\alpha/2 + Z\beta)^2 \times 2 \times \sigma^2/d^2$ (where, $Z\alpha/2 = 1.96\%$ at 95% confidence interval, $Z\beta = 2.33$ at 99% power of the study. $\sigma^2 = 677.211$, $d = 44.666$, $n = 12.494 = 13$ per group). A total of 39 participants were randomly divided into three groups with 13 participants each, after obtaining ethical clearance and informed consent. The registration number for this trial is CTRI/2023/05/053183.

Group A (negative control): Sterile water; Group B (positive control): 0.2% chlorhexidine mouth rinse; and Group C: 2.5% coffee pulp extract-based mouth rinse.

Saliva samples were collected from all the participants in sterile Eppendorf tubes before the use of mouth rinse to establish the baseline *S. mutans* colony count. A second sample of saliva was collected 1 h after the first rinse. The participants were instructed to rinse with 5 ml of the respective mouth rinse for 1 min twice daily for 2 weeks. The third sample was collected following 2 weeks use of the respective rinses and *S. mutans* colony count was estimated. The saliva samples were cultured on Mitis Salivarius Agar enriched with bacitracin, which is a selective media for *S. mutans*. The total count was evaluated using image-based software analysis.



Graph 1: Mean microbial count

The data were collected, coded, and fed in IBM SPSS (Horber, n.d.) for statistical analysis. The descriptive statistics included mean and standard deviation. The inferential statistics included one-way ANOVA and repeated measures ANOVA, followed by *post hoc* Tukey's test. The level of significance was set at 0.05 at 95% confidence interval.

RESULTS

A Tukey's *post hoc* test revealed a statistically significant reduction of the microbial count in the coffee pulp mouth rinse (5019.15 ± 1350.001) group ($P = 0.035$) and positive control (5023.31 ± 2538.318) group ($P = 0.036$) at 2 weeks postrinse compared to the negative control (6955.54 ± 1602.773).

A Tukey's *post hoc* test revealed that the difference in the microbial count reduction at 2 weeks postrinse was not statistically significant between coffee pulp mouth rinse (5019.15 ± 1350.001) and positive control (5023.31 ± 2538.318) with $P = 1$.

Repeated measures ANOVA determined that the mean microbial count did not differ significantly across the three different time intervals in negative (F [2,24] = 0.191, $P = 0.807$) as well as positive control groups (F [2,24] = 3.370, $P = 0.067$). Whereas, it differed significantly in the coffee pulp extract-based mouth rinse (F [2,24] = 6.776, $P = 0.006$).

A *post hoc* pairwise comparison using the Bonferroni correction showed a decreased microbial count from prerinse (6929.77) to 1-h postrinse (6202.38), but this was not statistically significant ($P = 0.477$). The *post hoc* test also did not show a significant ($P = 0.196$) decrease in the microbial count from 1 h postrinse (6202.38) to 2 weeks postrinse (5019.15). However, the decrease in microbial count did reach significance ($P = 0.007$) when comparing the prerinse (6929.77) to the 2 weeks postrinse (5019.15) [Graph 1].

DISCUSSION

Many previous studies on the antibacterial activity of coffee

focused on the use of coffee beans. Coffee, as a beverage, is shown to help in the prevention of dental caries if consumed without additives.[13] Nevertheless, the study of the antibacterial activity of the various by-products of coffee is seemingly limited. Various studies carried out to assess the composition of these by-products have shown the presence of active compounds. Coffee pulp extracts are known to have antibacterial action,[11] but their action against *S. mutans* has not been assessed clinically.

Safety of coffee pulp

A 2019 study suggested the possible protective effects of coffee cherry extracts on cells from oxidative stress. Furthermore, Hen's Egg Test on the Chorioallantoic Membrane model, which is used for predicting the irritation effect on the conjunctiva, confirmed the safety of the tested substance.[14]

A study in 2020 by Cañas *et al*. presented a critical evaluation of coffee pulp for its use in human food, ensuring this sustainable food ingredient's safety.[15] The European Food Safety Authority in its technical note of April 2021 reported that coffee pulp does not raise safety concerns considering the available data on its composition and history of use.[16]

A 2021 study evaluated the antioxidant status and antiaging properties of coffee pulp supplements at a concentration of 14% as a drink and a 2% concentration of serum application groups and the results were suggestive of reducing free radical activities, thereby delaying the skin aging process and enhancing skin health.[17]

The antimicrobial action of 2.5% coffee pulp mouth rinse

The mouthwash showed a decrease in antimicrobial activity, but other signs of deterioration such as agglomeration or change in clarity were not observed, after being subjected to the determination of stability to aging for 2 weeks at room temperature. Therefore, the participants were advised to refrigerate the mouth rinse after each use as the refrigeration helped prevent the deterioration of the antimicrobial properties of the rinse. The mouthwash was devoid of any readily fermentable carbohydrates. The pH of the mouth rinse was found to be at 6.9, which is well within the prescribed range between 3.0 and 10.5 by ISO 16408:2015.[18]

Saliva samples were collected in preference to plaque samples as a result of a more constant salivary microbial count than plaque, and the possible fluctuation in saliva microbial counts was controlled by sampling saliva at the same hour every day.[19]

The saliva samples were cultured on Mitis Salivarius Agar enriched with bacitracin, which is a selective media for *S. mutans*. The total count was evaluated using an image-based software analysis.

There was a significant reduction in the *S. mutans* count in the coffee pulp group ($P = 0.035$). This may be attributed to the chlorogenic acid and polyphenols, the primary constituents in coffee. Although the anticariogenic properties of coffee pulp have not been previously studied, the composition of coffee pulp comprises various actives such as tannins, pectic substances, caffeine, chlorogenic acid, and caffeic acid.[20] Chlorogenic acid is structurally composed of an ester of caffeic acid with the 3-hydroxyl group of quinic acid. Zhang *et al*. reported that the interaction between polyphenols and organic matrices leads to inhibition of the demineralization process.[21] This interaction involves covalent, ionic, hydrogen bonding, or hydrophobic processes, resulting in the alteration of the enamel organic matrix. Further, this altered organic matrix is precipitated in the enamel, resulting in reduced mineral ions loss, thereby inhibiting the demineralization of enamel. A study by Antonio *et al*., 2011, reported that a light-roasted *Coffea canephora* aqueous extract has potential anticariogenic effects due to its activity of preventing the growth of *S. mutans* and inhibiting dental demineralization.[22] Kashket *et al*. reported that polyphenols inhibit the formation of glucosyltransferase by cariogenic bacteria.[23] Antonio *et al*., 2010, suggested that caffeic acid and 5 caffeoylquine, along with polyphenols, showed some activity against *S. mutans*.[24]

In this study, the mean microbial count differed significantly across three time points ($P = 0.006$). No statistically significant difference was observed between prerinse and 1-h postrinse ($P = 0.477$) nor between 1-h postrinse and 2 weeks postrinse ($P = 0.196$). However, the decrease in microbial count did reach significance ($P = 0.007$) when comparing the prerinse to the 2 weeks postrinse, thereby showing a gradual decrease in microbial count over the duration of its use and suggesting its potential to be an anticariogenic agent.

The staining potential of coffee is attributed mainly to the polyphenolic compounds which provide the color in food.[25] Studies have shown the complex interactive effects between common drinks and salivary components that lead to staining. The effects of which are altered by mechanically or chemically induced changes in saliva deposition, suggestive of the complex mechanisms during the staining process.[26]

Limitations of the study

The quantity and quality of saliva are influenced by various factors such as diet, physical activity, seasons, and circadian rhythm. These factors can also contribute to the change in *S. mutans* count. A few of these factors were not standardized in this study.

Furthermore, this study aimed at assessing the reduction in the *S. mutans* colony count which is just one part of the multifactorial approach to the prevention of dental caries.

A major drawback seen in the case of coffee pulp mouth rinse was its bitter taste which could not be masked. It could reduce patient compliance.

CONCLUSION

Within the limitations of this study, it can be concluded that:

a. There was a significant reduction in the microbial count in the coffee pulp mouth rinse group ($P = 0.035$) and positive control group ($P = 0.036$) at 2 weeks postrinse compared to the negative control
b. There was no significant difference in the microbial count reduction between coffee pulp mouth rinse and positive control at 2 weeks postrinse with $P = 1$
c. The mean microbial count for the coffee pulp mouth rinse group differed significantly across three-time points ($P = 0.006$).
    - No statistically significant differences were observed between prerinse and 1-h postrinse ($P = 0.477$) nor between 1-h postrinse and 2 weeks postrinse ($P = 0.196$)
    - However, the decrease in microbial count did reach significance ($P = 0.007$) when comparing the prerinse to the 2 weeks postrinse.

The coffee pulp extract-based mouth rinse offers few advantages over chlorhexidine as no instances of staining, altered taste, or any allergic reactions were reported by the subjects. It can be considered a good at-home, caries preventive measure with naturally occurring active components, and possibly fewer adverse effects.

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Conflicts of interest

There are no conflicts of interest.

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