

**EVALUATION OF ANTIMICROBIAL ACTION OF COFFEE AND ITS BY-PRODUCTS
AGAINST STREPTOCOCCUS MUTANS: AN INVITRO STUDY****¹Dr. Keerthan Bollamma P. B., ²Dr. Nanjamma K. K. and ³Dr. Ponnappa K. C.**¹Post Graduate Student, Department of Conservative Dentistry and Endodontics, Coorg Institute of Dental Sciences, Virajpet.²Professor, Department of Conservative Dentistry and Endodontics, Coorg Institute of Dental Sciences, Virajpet.³Professor and Head, Department of Conservative Dentistry and Endodontics, Coorg Institute of Dental Sciences, Virajpet.***Corresponding Author: Dr. Keerthan Bollamma P. B.**

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ABSTRACT

Background & Objectives: Dental caries is widespread despite efforts made toward its prevention, the quest for a potentially more effective preventive agent with lesser adverse effects continues. Coffee, a local produce of Coorg in Karnataka, is one such herbal alternative. This study aims at assessing the potential antimicrobial activity of various Robusta coffee extracts on *Streptococcus mutans*. **Methods:** Extracts of green coffee bean, coffee pulp, and coffee leaves were prepared by various methods (boiling, maceration, Soxhlet extraction) and using different solvents (distilled water, ethanol, ethyl acetate, hexane) to determine the extraction procedure which gives adequate antimicrobial action against *Streptococcus mutans*. **Results:** Out of the samples evaluated for antimicrobial efficacy, only coffee pulp extracts using ethyl acetate and ethanol showed clear zones of inhibition in well cultures on Mitis salivarius agar enriched with bacitracin. The yield was greater when ethanol was used as a solvent, and hence it was used for microplating to determine the Minimal Inhibitory concentration which was 12.5 mg/ml concentration. **Interpretation & Conclusion:** Within the limitations of the study, it can be concluded that coffee pulp is a potential herbal alternative for caries prevention considering its antimicrobial action against *Streptococcus mutans*. Further studies are required to assess its efficacy invivo.

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 in 2019.^[1] With the progress of Minimal Invasive Dentistry, there has been an increased emphasis on caries prevention. The use of mouth rinses for the prevention of dental caries in children and adolescents was established as a mass prophylactic method with an average efficacy of caries reduction between 20-50%.^[2] 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.^[3] Chlorhexidine has also been reported to cause staining and transient impairment of taste perception.^[4] Though fluoride mouth rinses have generally proved to be effective in controlling caries in clinical studies, its benefits in adults have been less well documented.^[5] Also, when the dosage and use are not monitored carefully, it can have toxic effects.^[6]

Various natural products have been used for the prevention of the occurrence of dental caries. Studying the effects of herbal agents on dental caries has led researchers to one of the most effective ways of preventing dental caries due to its accessibility, low cost, and effectiveness. Coffee, a local produce of Coorg in Karnataka, India, is one such herbal alternative. Coffee is the second largest worldwide commodity, overshadowed only by crude oil, and is one of the most widely consumed beverages in the world. Coffee is a pharmacologically active tropical plant prompting numerous studies on its potential actions. Coffee is shown to have compounds that offer a variety of health benefits including antibacterial, antioxidant, anti-diabetic, and anti-inflammatory actions. Many previous studies on the antibacterial activity of coffee focussed on the use of coffee beans. Nevertheless, the study of the antibacterial activity of the various by-products of coffee is seemingly limited. Green coffee bean extract, which contains caffeine, volatile, and non-volatile organic acids such as chlorogenic acids (CGAs) and caffeic acids, have antibacterial properties,^[7] which is also effective against *Streptococcus mutans*.^[7,8] Coffee pulp and coffee leaf

extracts are known to have antibacterial properties,^[9,10] but their action against *S mutans* is yet to be evaluated. This study aims at assessing the potential antimicrobial activity of various Robusta coffee extracts against *Streptococcus mutans*.

METHODOLOGY

The freshly harvested coffee cherries and leaves & leaves were washed. The coffee bean and the coffee pulp were hand separated. The coffee leaves were washed and

dried. The samples were dried in an incubator at 40 degrees Celsius and then powdered.

The extracts were prepared using different methods like:

- Boiling 5 grams of the respective powdered sample (leaf, coffee pulp, green coffee bean) in 100 ml of distilled water.
- Maceration using solvents of various polarities such as distilled water, ethanol, ethyl acetate, and hexane.
- Soxhlet methods of extraction using distilled water, ethanol, ethyl acetate, and hexane.



Figure 1: Preparation of Coffee pulp extract by boiling, maceration and Soxhlet method of extraction.

All these extracts were then evaluated for their antimicrobial action against *S. mutans*.

[Bacterial strain used for the study: *Streptococcus mutans* Clarke (ATCC 25175)]

The prepared extracts were plated in a well culture of Mitis salivarius agar enriched with bacitracin. The extracts which showed clear zones of inhibition were then evaluated for the Minimum Inhibitory Concentration by serial dilutions and microplating.

RESULTS

Out of the samples evaluated for antimicrobial efficacy, only coffee pulp extracts using ethyl acetate and ethanol showed clear zones of inhibition. The quantity of the extract obtained was greater when ethanol was used as a solvent, and hence the ethanol extract of coffee pulp was continued in the study.

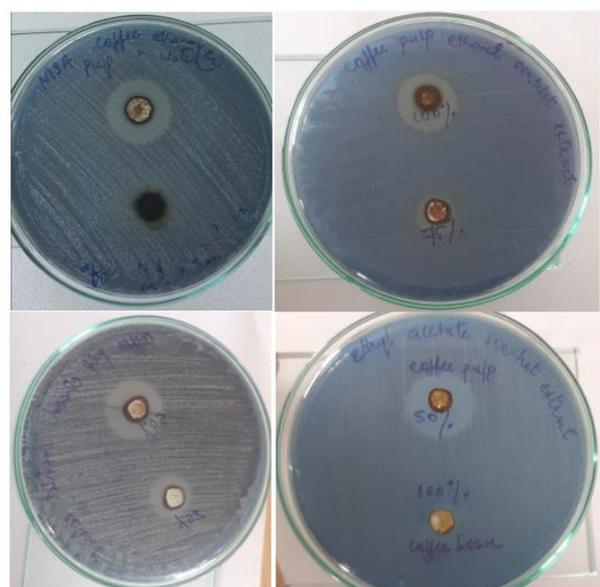


Figure- 2: Ethanol and Ethyl acetate-based Coffee Pulp extracts showing zones of inhibition.

The minimum inhibitory concentration of the extract was determined using microplating, the extract was not completely soluble in distilled water and hence a 5% DMSO was used. After serial dilutions and microplating, the MIC was determined to be 12.5 mg/ml.

DISCUSSION

When coffee is thought of, we generally associate with the bean alone; Even for coffee connoisseurs, it is unusual to think about the use of coffee leaves and pulp. Coffee pulp has also been used in beverages, Qishr, a southern Arabic traditional hot drink, made of spiced coffee husks, and a more recent western version of the beverage is cascara (the Spanish word for coffee husk). The use of coffee leaves for brewing tea referred to as “Kahwa Daun” or “Kawa” has long been a tradition in West Sumatra, Ethiopia, Jamaica, Java, India, and South Sudan.

Many previous studies on the antibacterial activity of coffee focus on the use of coffee beans. Nevertheless, the study of the antibacterial activity of the various by-products of coffee is seemingly limited. During agronomic management practice, the coffee leaves are pruned and thereby creating agricultural waste in the process. Coffee pulp is a by-product derived during wet processing of the coffee cherries. Various studies carried out to assess the composition of these by-products have shown the presence of active compounds. Coffee pulp and coffee leaf extracts are known to have antibacterial action^[9,10] but their action against *S. mutans* is yet to be evaluated. The study was initially designed to assess the antimicrobial action of green coffee bean, coffee pulp, and coffee leaf. But despite multiple efforts in making extracts using different methods and solvents, antimicrobial properties against *S. mutans* could not be established for green coffee bean extract and coffee leaf. This finding is not in accordance with studies conducted by M Yadav et al in 2017 and Gowtham et al in 2020, both of which showed antimicrobial property of green coffee bean extract.^[7,8]

Ethanol-based and ethyl acetate-based extracts of coffee pulp showed good antimicrobial effects against *S. mutans*. Ethanol when used as a solvent had a better yield and therefore was used in the rest of the study.

SAFETY OF COFFEE PULP

A 2019 study provided results suggestive of the possible protective effects of coffee cherry extracts on cells from oxidative stress. Also, an irritation test by Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) model confirmed the safety of the tested substance.^[11]

A study in 2020 by Canas et al presented a critical evaluation of coffee pulp for its use in human food, ensuring this sustainable food ingredient's safety.^[12] European Food Safety Authority (EFSA) 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.^[13]

A 2021 study evaluated the antioxidant status and anti-aging 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 skin aging process and enhancing skin health.^[14]

Coffee pulp comprises of various actives such as tannins, pectic substances, caffeine, chlorogenic acid, and caffeic acid.^[15] Chlorogenic acid is structurally composed of, an ester of caffeic acid with the 3-hydroxyl group of quinic acid. Zhang L et al., reported that the interaction between polyphenols and organic matrices leads to inhibition of the demineralization process by altering the organic matrix of enamel, resulting in reduced mineral ions loss, thereby inhibiting the demineralization of enamel.^[16] A study by Antonio et al, 2011, reported a light-roasted *C. canephora* aqueous extract has a potential anticariogenic effects due to its activity of preventing the growth of *S. mutans* and of inhibiting dental demineralisation.^[17] Kashket S et al. reported that polyphenols inhibit the formation of glucosyltransferase by cariogenic bacteria.^[18] Antonio AG et al., 2010, suggested that Caffeic acid and 5 caffeoylquinic, along with polyphenols, show some activity against *Streptococcus mutans*.^[19] Though the exact mechanism of action of coffee pulp extract is not completely understood yet, our study showed convincing results regarding its antimicrobial action against *Streptococcus mutans* thereby by suggestive of its anti-cariogenic potential.

CONCLUSION

Within the limitations of the study, it can be concluded that coffee pulp has antimicrobial action against *Streptococcus mutans*. This will allow coffee pulp to be a potential herbal alternative for caries prevention. And also, there are studies suggestive of its safe use. Further studies are required to assess its efficacy in vivo.

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