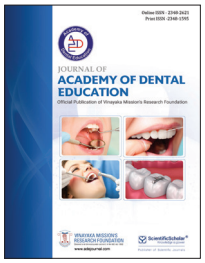
ScientificScholar®
Knowledge is power
Publisher of Scientific Journals

https://adejournal.com/

Journal of Academy of Dental Education



Original Article

A pilot study on the antimicrobial efficacy of *Coleus Aromaticus* on *Lactobacillus acidophilus* – Comparative *In-Vitro* analysis of five samples on a single plate

B. Sai Krishnan[1], Yadav Chakravarthy[1], Mathew Jacob[2]

[1]Intern, Departments of Conservative Dentistry and Endodontics and [2]Oral Pathology and Oral Microbiology, Vinayaka Mission's Sankarachariyar Dental College, Vinayaka Mission's Research Foundation (Deemed to be University),Salem Tamil Nadu, India.

***Corresponding author:**
Mathew Jacob,
Department of Oral Pathology and Oral Microbiology, Vinayaka Mission's Sankarachariyar Dental College, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India.

drmathewjacob@vmsdc.edu.in

Received: 02 January 2024
Accepted: 15 April 2024
EPub Ahead of Print: 30 May 2024
Published: 10 June 2024

DOI
10.25259/JADE_1_2024

Quick Response Code:

ABSTRACT

Objectives: *Coleus aromaticus*, also known as Indian borage, is a plant recognized for its medicinal uses, including potential antimicrobial properties. *Lactobacillus acidophilus*, a bacterium commonly found in the oral cavity and gastrointestinal tract, plays a crucial role in maintaining microbial balance but can lead to dental caries if imbalanced. The aim of this study is to assess the *in vitro* antimicrobial efficacy of *C. aromaticus* against *L. acidophilus*.

Material and Methods: The study was performed by the utilization of homeopathic ethanolic and dry leaf extracts of *C. aromaticus* on *Lactobacillus* bacteria, with a control group treated with 0.2% chlorhexidine. For the preparation of the *C. aromaticus* extract, leaves were cleaned, dried, and ground into a paste. A 20 g portion of this paste was then weighed and used for extraction. In the assessment of inhibitory effects, the disc diffusion method was employed, and nutrient agar served as the growth medium. Discs loaded with the respective extracts were positioned on the agar, and the Petri dishes were subsequently incubated at 37°C for 24 h. Following the incubation period, the inhibitory zones surrounding the discs were measured using a measuring scale.

Results: The study's findings were determined by measuring the diameter of inhibition zones and assessing the mean values. It was observed that the aqueous extract derived from dried *C. aromaticus* leaves possesses antimicrobial activity against *Lactobacillus*. Specifically, it exhibited inhibition zones with diameters of 24 mm and 27 mm when using 100 µL/disc and 200 µL/disc, respectively. In comparison, the control group (0.2% chlorhexidine) displayed an inhibitory zone with a 23 mm diameter.

Conclusion: The reason for these findings could be attributed to the existence of phytochemicals and essential volatile oils within *C. aromaticus*, which possess antimicrobial properties against *Lactobacillus*. This natural component appears to be effective in reducing *Lactobacillus* growth when used as an oral rinse containing phytochemicals.

Keywords: Coleus aromaticus, Lactobacillus acidophilus, Phytochemicals

INTRODUCTION

Dental caries is a prevalent chronic condition that gradually damages tooth tissues through the demineralization of enamel and dentin due to bacterial infection and dietary acids in dental plaque. Bacteria such as *Streptococcus*, *Actinomyces,* and *Lactobacillus* contribute to this condition, with *Lactobacillus* often linked to cavity formation, especially in children.[1]

Chlorhexidine is a commonly used mouthwash with antimicrobial properties. Its effectiveness varies depending on the concentration, with the ability to either inhibit bacterial growth (bacteriostatic) or directly kill bacteria (bactericidal). It works by penetrating the cell wall, causing cytoplasmic lysis and leading to cell death within 20 s of application.[2] Despite its advantages, 0.2% chlorhexidine has some drawbacks, including tooth staining, throat irritation, reduced taste sensation, potential gastric irritation, and the promotion of tartar formation. To address these limitations, many researchers have explored natural alternatives that offer safety, cost-effectiveness, and minimal to no side effects. Over the years, studies have focused on natural derivatives such as basil (Tulasi), *aloe vera*, neem, green tea, garlic solutions, and pomegranate as potential alternatives to 0.2% Chlorhexidine.[3,4]

The primary objective of this study is to develop a phytochemical-based therapeutic oral rinse designed to inhibit the growth of *Lactobacillus*, with a comparison to the most commonly used mouthwash, 0.2% chlorhexidine.

MATERIAL AND METHODS

The study was approved by the Institutional Ethics Committee, 27th April 2023, Ethical approval number (IEC/ Approval No.304). All procedures performed in the study were conducted in accordance with the ethical standards given in 1964 Declaration of Helsinki, as revised in 2013.

Study design

The present study was an *in vitro* study and the sampling method utilized in the study was convenience sampling.

Study samples

C, A1, A2, B1, and B2 represent different treatment groups. The details of the sample and the concentration of treatment are;

- C: Control 0.2% chlorhexidine mouthwash IP 200 µL
- A1: Leaf sample, *Coleus aromaticus*, was applied at a concentration of 100 µL
- A2: Leaf sample – *C. aromaticus* was applied at a concentration of 200 µL
- B1: Homeopathic extract *C. aromaticus* was applied at a concentration of 100 µL
- B2: Homeopathic extract *C. aromaticus* was applied at a concentration of 200 µL.

Study setting

The study was conducted in the Department of Conservative Dentistry and Endodontics.

Methodology

The *C. aromaticus* extract was prepared by washing, drying, and grinding the leaves into a paste, followed by weighing 20 g of the paste and extracting it using water as a solvent. Homeopathic ethanolic extracts were diluted to various concentrations for antimicrobial testing and all extracts were stored individually. The disc diffusion method was utilized to determine inhibitory zones, with nutrient agar as the growth medium. The plates, seeded with bacteria, were incubated at 37°C for 24 h and the growth response to different extracts was observed. Antimicrobial activity was calculated as the mean zone of inhibition against *Lactobacillus*, with 0.2% chlorhexidine as the control group. The antimicrobial susceptibility test involved preparing nutrient agar plates, swabbing them with a 0.1% inoculum suspension, placing sterile discs loaded with extract concentrations, and incubating them at 37°C for 24 h. The concentrations of extracts are 100 µL/disc and 200 µL/disc were loaded on sterile discs. Following incubation, the inhibitory zones around the discs were measured using a scale.

Statistical analysis

The inhibitory zones from the samples of raw leaves and extracts were measured and compared with the control.

RESULTS

The inhibitory zone diameter in the control group (chlorhexidine mouthwash IP), A1 at 100 µL, A2 at 200 µL, B1 at 100 µL, and B2 at 200 µL was found to be 23, 24, 27, 17, 22 mm, respectively, as shown in Figure 1. Table 1 indicates

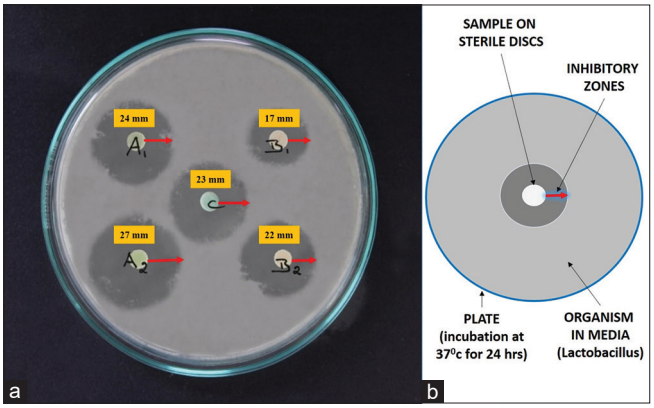


Figure 1: (a) The single plate with five samples (A1, A2, B1, B2, C) at different concentrations with the respective measurement of zones of inhibition (Red arrows), (b) Schematic showing the parts of the plate and measurements taken.

Table 1: The inhibition of *Lactobacillus* growth in each treatment.

Organism	Measurement (in mm)				
	Control	A1	A2	B1	B2
Lactobacillus	23	24	27	17	22

that the inhibitory effect on *Lactobacillus* varies among the treatments.

The varying degrees of antibacterial activity among the different treatments, with treatment with A2 showing the strongest inhibition, followed by the control group (chlorhexidine mouthwash IP). Treatment B2 demonstrated moderate inhibitory activity, while both treatments A1 and B1 exhibited relatively weaker inhibition.

DISCUSSION

Dental caries, a prevalent and chronic ailment, is characterized by the gradual deterioration of enamel, dentin, and cementum, leading to the decalcification of these tissues and the breakdown of organic substances. This infection arises from the demineralization of enamel and dentin due to the action of organic acids produced by bacteria found in dental plaque through anaerobic metabolism, playing a pivotal role in the development of dental caries. Among these bacteria, *Lactobacillus* is particularly noteworthy as a major contributor to dentinal caries. Lactobacilli are closely linked to the advancement of lesions, primarily observed in rampant caries. Evidence suggests that acidophilus, a microorganism known for its significant acid-producing and acid-tolerant characteristics, plays a pivotal role in the development of dental caries.[5,6]

Chlorhexidine (0.2%) is commonly employed as a mouthwash for the prevention of bacterial and microbial growth. Its effectiveness varies depending on the concentration used, as it can exhibit both bacteriostatic and bactericidal effects. It permeates the cell wall, leading to cytoplasmic lysis and cell death within a mere 20 s of application. Despite its numerous advantages, chlorhexidine is not without its drawbacks, which include tooth staining, throat irritation, reduced taste sensation, gastric discomfort, and the promotion of tartar formation.[7]

To address these limitations, researchers have explored natural alternatives that are not only safe and cost-effective but also associated with minimal to no side effects. Over the years, various natural derivatives such as Tulasi, *aloe vera*, neem, green tea, garlic solutions, and pomegranate have been studied for their potential benefits. Despite the widespread use of chlorhexidine, there is a continuous preference for alternative, natural or organic mouth rinses. In our study, we specifically investigated *C. aromaticus* due to its multifaceted effects and its minimal impact on side effects. *C. aromaticus*, a member of the *Lamiaceae* family, is also known as Mexican mint and is locally referred to as "karpuravalli." It possesses significant antimicrobial and antibacterial properties, along with bacteriostatic and fungistatic qualities. Previous research has delved into the antibacterial and anticancer potential of extracts derived from its leaves.[8]

The purpose of this study was to develop a phytochemical therapeutic oral rinse capable of inhibiting the growth of *Lactobacillus* in comparison to the most commonly used mouthwash, 0.2% chlorhexidine. In the control group, where 0.2% chlorhexidine Mouthwash IP served as the reference or standard treatment, the inhibitory zone diameter was 23 mm, consistent with findings from previous studies involving other microorganisms.

Using a leaf sample of *C. aromaticus* at a concentration of 100 µL, the inhibitory zone diameter for this treatment measured 24 mm, indicating a slightly larger inhibition zone compared to the control. However, when the *C. aromaticus* leaf sample was applied at a higher concentration of 200 µL, it produced a significantly larger inhibitory zone with a diameter of 27 mm, surpassing both the control and the lower concentration treatment. This suggests that the higher concentration of the *C. aromaticus* leaf sample possesses a more potent antibacterial effect against *Lactobacillus*. This could be probably due to the presence of phytochemicals present in the leaves. The antibacterial effect of *C. aromaticus* was probably due to the presence of phytochemicals, volatile oil compounds, phenolic compounds, etc.

C. aromaticus contains numerous volatile oils and cells that secrete essential oils, each with a diverse range of chemical compositions. It also includes phenolic substances, primarily made up of eugenol, methyl eugenol, and thymol, which make up a significant portion of its phytochemical composition. It is important to note that these specific constituents are particularly prevalent in *C. aromaticus* found in the Indian region, specifically the variety containing Wild Oregon, as highlighted by authors Baslas and Kumar in 1981. Furthermore, despite the extensive array of phytochemicals and volatile oils present in *C. aromaticus*, there are noteworthy concentrations of y-terpinene and p-cymene. These components are of particular interest because they serve as precursors for thymol and carvacrol, as explained by Prudent.[9,10]

The preference for using extracts lies in the ability to produce higher concentrations, thus potentially increasing efficacy and obtaining a more purified form that minimizes unintended side effects resulting from impurities. When *C. aromaticus* extracts were employed at a concentration of 100 µL, the inhibitory zone diameter was 17 mm, notably smaller than that observed with 0.2% chlorhexidine mouthwash IP. This indicates that the homeopathic extract at this concentration exerts a weaker antibacterial effect compared to 0.2% chlorhexidine mouthwash IP. Similarly, when the extract was used at a higher concentration of 200 µL, the inhibitory zone diameter measured 22 mm, reflecting a larger inhibition zone. This implies that the higher concentration of the homeopathic extract has a relatively stronger antibacterial effect than the lower concentration and underscores the

variation in inhibitory effects on *Lactobacillus* based on the raw material and concentration of the extracts.

As dental caries continue to increase among both children and adults, ongoing research aims to develop a vaccine capable of preventing or reducing caries incidence. Such a vaccine would offer significant benefits, especially for individuals with intellectual disabilities and those suffering from systemic diseases that compromise their immunity, such as type 2 diabetes. However, it is important to acknowledge that developing such a vaccine is a complex and lengthy process. In this context, natural or organically derived mouth rinses emerge as an alternative to synthetic rinses. They hold the potential to address concerns related to antimicrobial resistance, which can be exacerbated by the use of synthetic products. In addition, these natural rinses may help maintain a healthy balance of normal oral commensals within the oral cavity while providing effective oral care.[11-13]

In this study, a cost-effective culture method was employed by consolidating five samples onto a single plate to assess the inhibitory growth of chlorhexidine, guided by the literature review indicating an inhibition zone of 23 mm. In summary, the study suggests that *C. aromaticus* extract could be a valuable and accessible resource for oral health in rural India, where microbial control is vital in preventing dental caries, and it offers potential benefits with fewer adverse effects compared to chlorhexidine. This finding may lead to the development of cost-effective and sustainable oral health interventions in these communities.

CONCLUSION

The prevention of dental caries primarily hinges on the management of microbial growth. Within the constraints of this study, it can be inferred that *C. aromaticus*, extracted from dried leaves in the form of an aqueous extract, demonstrated superior antimicrobial efficacy against *Lactobacillus* when compared to 0.2% chlorhexidine mouthwash and oral rinse. The main implication of this research is its potential utility in school-based preventive oral health programs in rural India, where *C. aromaticus* leaves are readily available and cultivated naturally and are associated with minimal to no adverse effects on the oral cavity.

Ethical approval

The research/study approved by the Institutional Review Board at Vinayaka Mission's Sankarachariyar Dental College, number IEC/Approval No.304, dated 27.04.2023.

Declaration of patient consent

Patient's consent is not required as there are no patients in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

1. Mathur VP, Dhillon JK. Dental caries: A disease which needs attention. Indian J Pediatr 2018;85:202-6.
2. Autio-Gold J. The role of chlorhexidine in caries prevention. Oper Dent 2008;33:710-6.
3. Krupa NC, Thippeswamy HM, Chandrashekar BR. Antimicrobial efficacy of xylitol, probiotic and chlorhexidine mouth rinses among children and elderly population at high risk for dental caries-A randomized controlled trial. J Prev Med Hyg 2022;63:E282-7.
4. Thomas A, Thakur SR, Shetty SB. Anti-microbial efficacy of green tea and chlorhexidine mouth rinses against *Streptococcus mutans*, Lactobacilli spp. and *Candida albicans* in children with severe early childhood caries: A randomized clinical study. J Indian Soc Pedod Prev Dent 2016;34:65-70.
5. Jay P. *Lactobacillus acidophilus* and dental caries. Am J Public Health Nations Health 1938;28:759-61.
6. Helman EZ, Mitchell DF. Lactobacilli and dental caries in the hamster. J Dent Res 1953;32:596-600.
7. Narayanan M, Vigneshwari P, Natarajan D, Kandasamy S, Alsehli M, Elfasakhany A, *et al*. Synthesis and characterization of TiO_2 NPs by aqueous leaf extract of *Coleus aromaticus* and assess their antibacterial, larvicidal, and anticancer potential. Environ Res 2021;200:111335.
8. Chandra Shekar BR, Nagarajappa R, Jain R, Singh R, Thakur R, Shekar S. Antimicrobial efficacy of *Acacia nilotica*, *Murraya koenigii* (L.) Sprengel, *Eucalyptus hybrid*, *Psidium guajava* extracts and their combination on *Streptococcus mutans* and *Lactobacillus acidophilus*. Dent Res J (Isfahan) 2016;13:168-73.
9. Baslas RK, Kumar P. Chemical examination of the essential oil of *Coleus aromaticus* Benth. J Indian Chem Soc 1981;58:103-4.
10. Prudent D. Analysis of the essential oil of wild oregano from Martinique (*Coleus aromaticus* Benth.)-evaluation of its bacteriostatic and fungistatic properties. J Essent Oil Res 1995;7:165-73.
11. Caldwell J, Lehner T. Immunisation of rhesus monkeys with *Streptococcus mutans*, *Lactobacillus acidophilus* and lipoteichoic acid for protection against dental caries. J Med Microbiol 1982;15:339-50.

12. Everhart DL, Mallett CP, Doyle G. Dental caries vaccine: Some problems solved? Microbiol Sci 1985;2:312-3.
13. Lehner T. Scientific basis for vaccination against dental caries. Proc Finn Dent Soc 1983;79:62-70.

How to cite this article: Sai Krishnan B, Chakravarthy Y, Jacob M. A pilot study on the antimicrobial efficacy of *Coleus Aromaticus* on *Lactobacillus acidophilus* – Comparative *In-Vitro* analysis of five samples on a single plate. J Academy Dent Educ. 2024;10:20-4. doi: 10.25259/JADE_1_2024