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ANTIBACTERIAL ACTIVITY OF COMMERCIAL ORAL HYGIENE PRODUCTS AGAINST ORAL BACTERIA AND THE OPPORTUNISTIC PATHOGEN *E. COLI*

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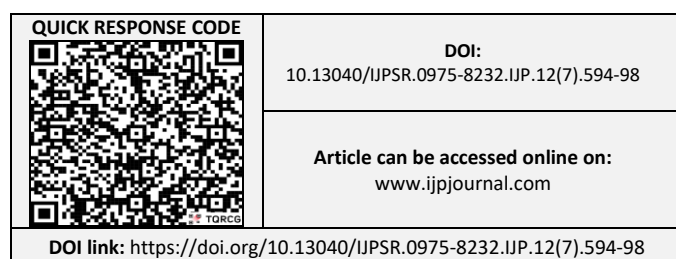
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ABSTRACT: This study evaluated the antibacterial activity of eight commercially available toothpaste samples (P1–P8) and a standard antibiotic control (Tetracycline) against three oral bacterial isolates (C2, C3, C4) and *Escherichia coli* using the well diffusion method. Zones of inhibition were measured to assess antimicrobial efficacy. Among all samples, P1 demonstrated the highest activity, with inhibition zones surpassing those of the antibiotic control for several strains. P8 exhibited consistent activity across all bacteria, while P2 was selectively effective against C3. In contrast, P4, P6, and P7 showed no activity against *E. coli*. Notably, *E. coli* resistance to several formulations underscores the need for rigorous post-brushing rinsing to minimize potential impacts on gut microbiota. The results affirm that antibacterial efficacy varies widely among commercial toothpaste brands, likely due to differences in formulation and active components. This study highlights the superior antibacterial action of certain commercial toothpastes compared to antibiotics and also underscores the importance of product-specific evaluation for effective oral health management.

INTRODUCTION: Bacteria are ubiquitous, microscopic, single celled organisms that colonize a range of environments including soil, oceans, and the human body. Within humans, bacteria can be beneficial, aiding indigestion and fermentation processes, or harmful, contributing to disease. In the oral cavity, bacteria form a complex ecosystem with over 300 different species identified. These bacteria initiate the digestion of food and interact with host tissues and other microbes. Notably, only a limited proportion of oral bacteria extend into the gastrointestinal tract, emphasizing the uniqueness of the oral environment ¹.

Tooth decay and plaque formation are primarily driven by the metabolic activities of oral bacteria. Plaque is a biofilm composed of bacteria embedded in an organic matrix. These microbes metabolize dietary sugars to produce acids that demineralize tooth enamel, leading to dental caries and periodontal disease ². Preventive measures such as mechanical brushing and the use of antimicrobial toothpastes and mouthwashes are essential for maintaining good oral health.

Toothpaste, a common dentifrice, is considered a medicinal product, rather than a cosmetic, due to its active ingredients that contribute to oral health ³. Active compounds include fluoride, antibacterial agents such as triclosan and chlorhexidine, desensitizing agents, anti-tartar agents, and enzymes. Fluoride strengthens enamel by forming fluoro-hydroxyapatite, which resists acid dissolution ⁴. Triclosan, a broad spectrum



antimicrobial, disrupts bacterial membranes without causing staining, unlike chlorhexidine⁵. Despite their efficacy, some studies suggest that fluoride only toothpastes have limited impact on reducing bacterial load^{6,7}. Additionally, traditional oral hygiene practices involving natural substances such as plant leaves and ashes remain in use, particularly in rural communities⁸.

Previous studies have produced mixed results regarding the antimicrobial efficacy of commercial toothpastes^{9,10}. While some formulations significantly reduce oral flora, others have limited or even adverse effects. Varied outcomes have been reported among ten toothpaste brands and the highest efficacy demonstrated by Colgate, although natural agents such as guava and mango leaves have shown antimicrobial properties *in-vitro*; comprehensive comparative studies remain limited¹¹.

Despite the widespread use of commercial and natural oral hygiene products, the comparative effectiveness of different toothpaste brands and traditional materials in inhibiting oral bacteria remains insufficiently explored, particularly in the context of commonly used brands in local markets. While fluoride and triclosan containing toothpastes have shown antimicrobial properties, their varying effectiveness across bacterial strains, including oral flora and opportunistic gut bacteria such as *Escherichia coli*, warrants deeper investigation. This study aims to evaluate the antibacterial potential of various commercial toothpaste brands and contributes to informed decision making in public health and dental hygiene practices.

MATERIALS AND METHODS:

Collection of Tooth Paste Brands: Eight commercially available toothpaste brands commonly used by consumers were procured from local retail outlets in Kottayam District, Kerala, India.

Bacterial Strains: Three oral bacterial strains (designated C2, C3, and C4) were isolated from the tooth surface of a human subject using sterile cotton swab. The swab was gently rubbed back and forth across the surface of the tooth to collect the microbial sample. The swab was then immersed in 9 mL of sterile saline solution to create a 10^{-1}

dilution, which was mixed thoroughly. Subsequently, serial tenfold dilutions were prepared up to 10^{-5} by transferring 1 mL of each dilution into 9 mL of fresh sterile saline. Bacterial isolation was performed using the spread plate technique. Aliquots of 0.1 mL from the 10^{-3} to 10^{-5} dilutions were aseptically spread onto sterile Nutrient Agar plates using a sterile L-shaped glass spreader.

The plates were incubated at 37°C for 24 hours. Distinct colonies were selected and purified by streaking onto fresh Nutrient Agar plates to obtain pure cultures. The culture of *E. coli* was procured from School of Biosciences, M.G. University, Kottayam, Kerala, India. The pure cultures of the bacteria were maintained on Nutrient Agar slants and stored at 4°C for further analysis.

Antimicrobial Assay by Well Diffusion Method:

Pure isolated colonies of each oral bacterial strain grown on Nutrient Agar were inoculated into sterile peptone water and incubated at 37°C for 24 hours. A 0.2 mL aliquot each of the resulting broth culture was uniformly spread onto the surface of Mueller–Hinton Agar (MHA) plates using a sterile cotton swab to create a lawn culture. Wells of 7 mm diameter were aseptically punched into the agar using a sterile gel borer. Each well was loaded with 50 μ L of the tooth paste samples. Tetracycline antibiotic discs were included on the culture plate of each bacteria as a positive control. The plates were incubated at 37°C for 24 hours and the antibacterial activity of each test sample was evaluated by measuring the diameter of the zone of inhibition around each well or disc to the nearest millimeter.

RESULTS: The antibacterial activity of eight commercial toothpaste samples (P1–P8) and a standard antibiotic control (Tetracycline, T) was evaluated against three oral bacterial isolates (C2, C3, C4) and *E. coli* using the well diffusion method. The diameter of the zone of inhibition was measured in millimeters, and the results are summarized in **Table 1**.

Among all tested samples, P1 exhibited the highest antibacterial activity, with inhibition zones of 30 mm (C2), 50 mm (C3), 35 mm (C4), and 38 mm (*E. coli*) diameter, surpassing even the standard

antibiotic in some cases. P2 showed strong activity against C3 (50 mm) but was ineffective against C2 and *E. coli*, indicating selective efficacy. Other samples such as P3, P4, P5, and P6 demonstrated moderate inhibitory effects, particularly against C3 and C4, but exhibited limited or no activity against *E. coli*. Toothpaste P8 showed consistent and balanced activity against all tested bacteria, with zones ranging from 23mm to 26mm. Samples P4, P6, and P7 were ineffective against *E. coli*, showing complete resistance (R). Tetracycline (T), used as a positive control, produced inhibition zones of 20 mm to 30 mm across all test organisms.

TABLE 1: ANTIMICROBIAL ACTIVITY OF TOOTH PASTE SAMPLES AGAINST ORAL BACTERIA AND *E. COLI*

Tooth Paste sample type	Diameter of zone of inhibition in mm for the various test bacteria			
	C2	C3	C4	<i>E. coli</i>
P1	30	50	35	38
P2	R	50	20	R
P3	15	22	28	13
P4	15	20	20	R
P5	20	25	22	20
P6	25	20	21	R
P7	20	22	21	R
P8	26	26	25	23
T	20	30	30	22

Key: R–Resistant (no zone of inhibition).

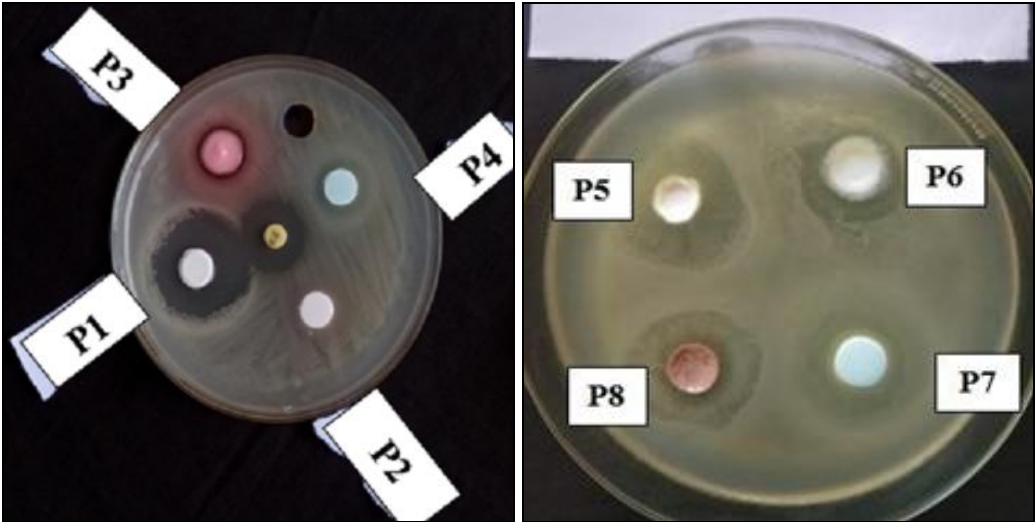


FIG. 1: ANTIBACTERIAL ACTIVITY OF TOOTH PASTES AGAINST THE ORAL BACTERIAL ISOLATE C2

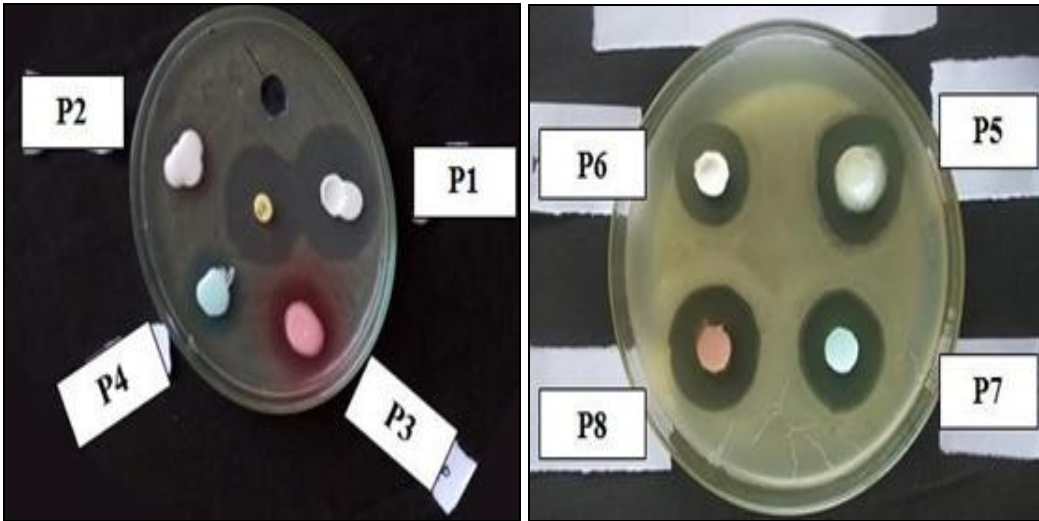


FIG. 2: ANTIBACTERIAL ACTIVITY OF TOOTH PASTES AGAINST THE ORAL BACTERIAL ISOLATE C3

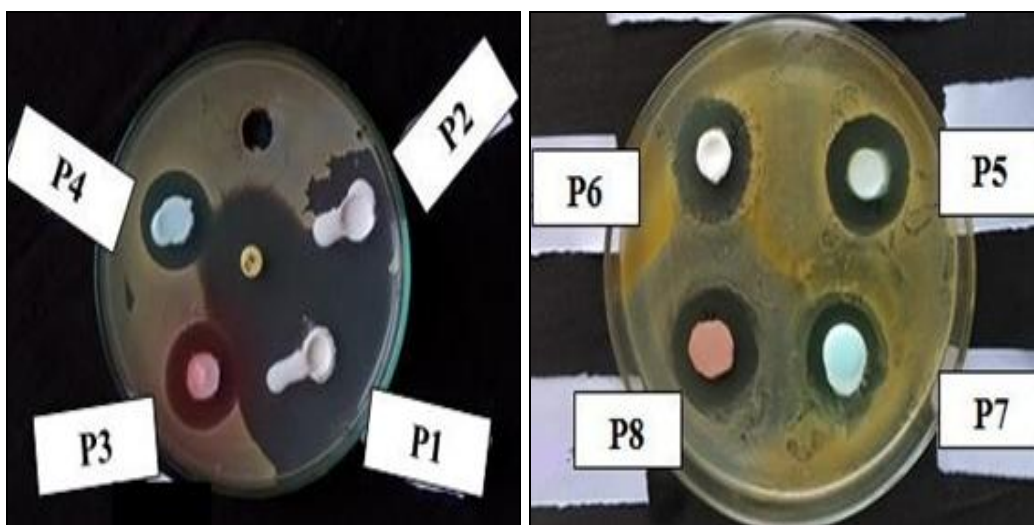
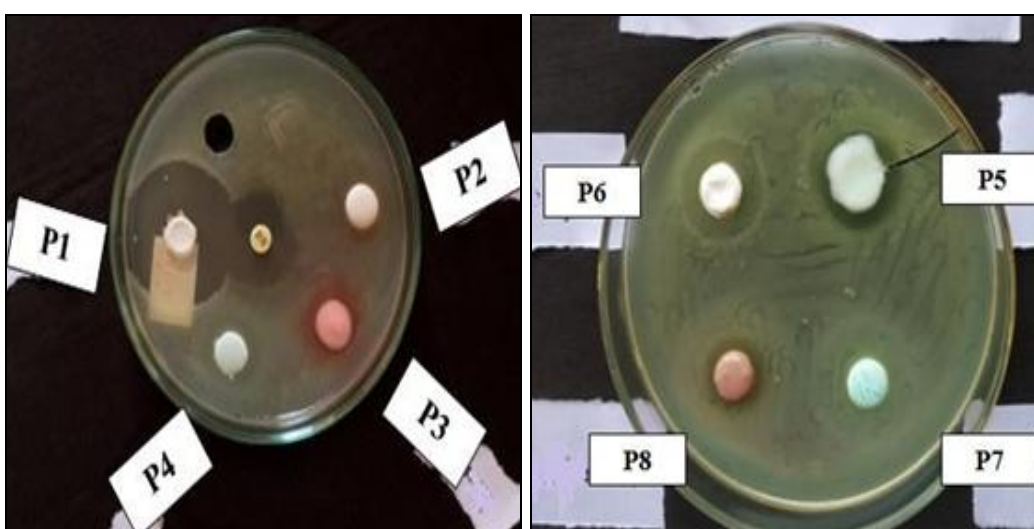


FIG. 3: ANTIBACTERIAL ACTIVITY OF TOOTH PASTES AGAINST THE ORAL BACTERIAL ISOLATE C4

FIG. 4: ANTIBACTERIAL ACTIVITY OF TOOTH PASTES AGAINST *E. COLI*

DISCUSSION: This study demonstrates that commercial toothpaste brands vary in antibacterial efficacy, with the sample P1 outperforming others. The superior performance of P1 suggests the presence of more potent antimicrobial agents or optimal formulation. Notably, *E. coli* was resistant to P2, P4, P6, and P7, emphasizing the importance of thorough rinsing after brushing to avoid potential disruption of gut microbiota. Overall, the results of this study indicate that some commercially available toothpaste possess significant antibacterial properties. However, variation in efficacy among products and bacterial resistance to certain formulations highlight the importance of product-specific evaluation for effective oral antimicrobial action. The observed inhibition of oral isolates aligns with previous studies which reported toothpaste-mediated reduction in oral bacterial load^{9, 11}. In contrast,

Okpalugo *et al.* (2009) and Oyarekua *et al.* (2015) reported increased bacterial counts post usage, possibly due to ineffective formulations or microbial contamination^{10, 12}. Fluoride only toothpastes have shown limited effectiveness in bacterial control as corroborated here^{6, 7}. Further, inclusion of *E. coli* as a test organism serves a dual purpose: it simulates possible accidental ingestion and offers insights into broader microbial impacts of toothpaste constituents.

CONCLUSION: This study demonstrates the superior antibacterial efficacy of certain commercially available tooth pastes, particularly sample P1, against oral bacteria and *E. coli*. These results highlight the importance of selecting effective oral hygiene products and raising awareness of potential systemic implications resulting from accidental ingestion.

Future research should focus on the molecular identification of the isolated bacterial strains, evaluate a wider range of oral pathogens, analyze the active chemical components of the most effective formulations, and investigate potential synergistic effects between natural extracts and commercial toothpaste products.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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