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ORIGINAL RESEARCH

Investigation on the antioxidant, antibacterial, and antibiofilm properties of three distinct herbal tooth powders

A. S. Smiline Girija[1], Pachamuthu Balakrishnan[1], Shanmugam Saravanan[1], R. Aravindhan[2], P. Selvam[2] and Rajesh Kanna Gopal[1]*

[1]Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India
[2]R&D Center, Aravindh Herbals Pvt. Ltd., Rajapalayam, India

***Correspondence:**
Rajesh Kanna Gopal,
rajeshkannag.sdc@saveetha.com

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Introduction: Oral health is entirely dependent on removing dental plaque on the tooth surface formed by various unique micro-organisms. Herbal toothpaste is equivalent to non-herbal fluoride toothpaste in removing dental plaque. Additionally, herbal toothpaste is advantageous in the strengthening of gums.
Methods: In this study, three herbal tooth powders, Brindha tooth powder (BTP), Red tooth powder (RTP), and Kosali tooth powder (KTP), were tested for antioxidant, antibacterial, and antibiofilm activities against clinical isolates.
Results: Brindha tooth powder (BTP) has the greatest free-radical scavenging percentage of 52.78%, followed by 40.9% for RTP, and 39.2% for KTP. Comparatively, RTP and BTP showed significant inhibition of *Enterococcus faecalis*, *Streptococcus mutans*, and *Pseudomonas aeruginosa* at three different concentrations (10, 5, and 2.5 mg). Among them, RTP showed a higher antibacterial effect with no inhibitory effect by KTP. However, antibiofilm activity was not observed with the toothpaste tested.
Conclusion: The study findings revealed promising and varying degrees of antioxidant and antimicrobial properties of the three different tooth powders.

Keywords: herbal tooth powder, antioxidant activity, antimicrobial activity, antibiofilm activity

1. Introduction

It been estimated that 3,500 million people are affected by untreated oro-dental diseases due to poor oral hygiene, but it was neglected in the global health agenda (1). Thus, the World Health Organization has recently adopted a resolution on the promotion of global oral health (2). Approximately 700 different kinds of microbes have been reported in the human oral microbiota, including pathogenic and probiotic species; the most common organisms found in the oral cavity are *Firmicutes*, *Fusobacteria*, *Gracilibacteria*, *Saccharibacteria*, *Chlamydiae*, *Bacillus*, *Actinomycetes*, *Proteobacteria*, *Spirochaetes*, and *Synergestitis* (3). Some of the oral pathogens are *Candida albicans,* which forms biofilm along with *Streptococcus mutans* causing dental plaque (4), *Porphyromonas gingivalis* (Gingivitis), *Lactobacillus* (Tooth-caries) (5, 6), and *Staphylococcus aureus* (7). Other anaerobic pathogens include *Atopobium*, *Leptotrichia*, *Lactobacillus salivarius*, and *Prevotella* (8).

Oral pathogens were also reported to mediate other systemic diseases such as endocarditis (9), rheumatoid arthritis (10), and some other autoimmune diseases. Oral pathogens are now understood to play a vital role in the pathogenesis of several oral disorders, including oral cancer, endodontic infections, dental caries, and periodontal diseases. In some cases, the virulence

of pathogenic oral microbes is enhanced in patients affected with diabetes (11). Through the esophagus, the oral microbiota directly enters the digestive system, disrupting the intestinal micro-ecology and altering the digestive system. Tooth caries is a general condition that infects people of all age group and has a higher rate of incidence. Children are more likely than adults to develop dental caries, which is intimately tied to the oral microbiome. According to a prior study, youngsters who frequently eat sweets before bed are more likely to develop dental caries.

Antibiotic treatment is effective but antimicrobial resistance (AMR) is triggered among such pathogens in hospitals and is an alarming threat to global health (12). Therefore, effective removal of dental plaque is required to maintain proper oral hygiene (13).

Toothpaste plays an important role in removing pathogenic microbes in our oral cavity in our daily life and improving our quality of life (14). Herbal tooth powder or plant extracts are used in rural villages of South Asian countries and are reported to possess antipyretic, analgesic, antibacterial, antiviral, antioxidant, anti-carcinogenic, and anti-inflammatory properties (15, 16). In addition to this, herbal toothpaste is effectively equivalent to non-herbal fluoride toothpaste in removing dental plaque and strengthening the gums (17–19).

Therefore, in the present investigation, three different herbal tooth powders, Red tooth powder (RTP), Brindha tooth powder (BTP), and Kosali tooth powder (KTP), were tested for their antioxidant and antimicrobial activity on three different clinical isolates of *Enterococcus faecalis* (EF), *Streptococcus mutans* (SM), and *Pseudomonas aeruginosa* (PE).

2. Materials and methods

2.1. Evaluation of antioxidant activity (DPPH free-radical scavenging assay)

The antioxidant potential of the samples Brindha tooth powder (BTP), Red tooth powder (RTP), and Kosali tooth powder (KTP) (Aravindh Labs Pvt. Ltd.) was determined based on the DPPH assay (20). About 100 μL of the test samples each was taken in separate wells in a microtitre plate, added with the same volume of DPPH (in 0.1% methanol), and incubated for 30 min under dark conditions. Ascorbic acid (Vitamin C) was used as a control. After incubation, the change in color of the solution was observed at 520 nm in an ELISA plate reader and the absorbance (ABS) values were recorded. The percent DPPH radical scavenging activity was calculated as [(ABS of ctrl–ABS of the test sample)/(ABS of ctrl)] × 100. The tests were done in triplets, and the average mean was interpreted and resulted.

2.2. Determination of *in vitro* antimicrobial activity

About 1 g samples of BTP, RTP, and KTP were extracted with 100 mL of 100% methanol for 2 days in a glass conical flask. After filtering the crude solvent with Whatman No. 1 filter paper, the filtrate was left to sit in an incubator overnight at 40°C to allow the solvent to evaporate and produce the extract. 200 mg samples of BTP, RTP, and KTP extracts were diluted and vortexed in 1 mL of sterile DMSO for analysis. Three different concentrations of the extracts were prepared for the study, viz., 10, 5, and 2.5 mg.

For the antimicrobial bio-assay, fresh broth suspensions were prepared for the test microorganisms up to 0.5 McFarland standards. The test microorganisms chosen for the study were clinical strains of *Pseudomonas aeruginosa* (Gram-negative) (PA) and *Enterococcus faecalis* (EF). Briefly, the test microorganisms were made as lawn cultures onto sterile Mueller Hinton (MH) agar plates, and wells were cut using a sterile agar cutter. Next, 50 μL extracts at three different concentrations were added to the corresponding wells. After incubation at 37°C for 24 h, the ZOI (clear zone) in and around the wells was measured and recorded. The tests were done in triplets, and the average mean was interpreted and recorded.

2.3. Determination of antibiofilm activity

Before the commencement of the assay, fresh MH broth suspension of test microorganisms was prepared up to 0.5 McFarland Standards. Then the 96-well plate was loaded with MH broth, sample, and inoculum to reach a total volume of 200 μL per well (80 μL broth + 100 μL test sample + 20 μL inoculum) (Rows A to F at different concentrations from 5 to 0.1 5 mg). Row G is control, and H is sterile control, where the former had 180 μL of broth and 20 μL of inoculum, and the latter contained 200 μL of the broth alone. The test microorganisms involved in this study were *Enterococcus faecalis* (EF), *Streptococcus mutans* (SM), and *Pseudomonas aeruginosa* (PE). The complete design of the antibiofilm assay for this study is clearly illustrated in **Figure 1**. After incubation for 48 h in an incubator at 37°C, the wells were washed with dis. H_2O, and each well received 50 μL of 0.1 percent crystal violet staining solution, which was then incubated for 10 min. The wells were washed with dis. H_2O and about 100 μL of 70% ethanol was added to each well and again incubated for 5 min. Finally, an ELISA plate reader was used to measure the absorbance values at 595 nm.

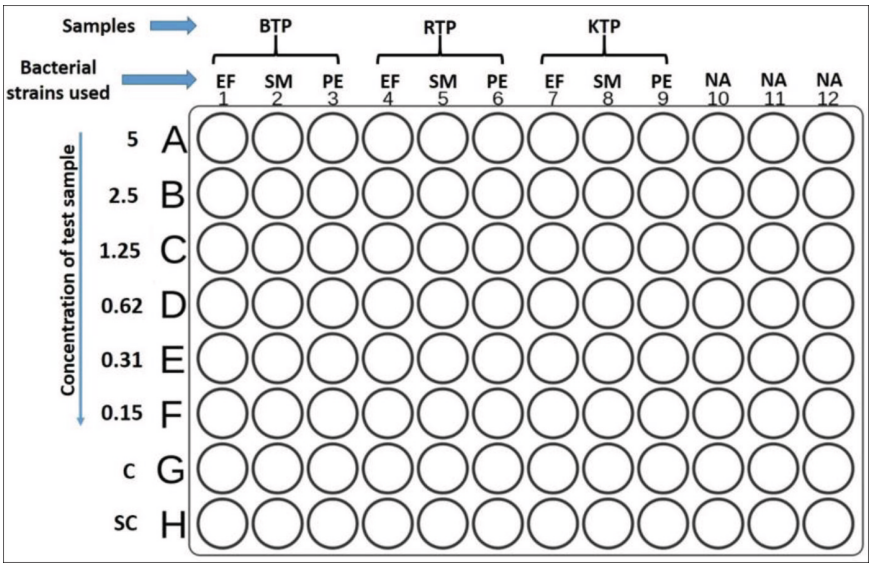

FIGURE 1 | A template of a 96-well plate in which the antibiofilm assay was designed, where, BTP: Brindha tooth powder; RTP: Red tooth powder; KTP: Kosali tooth powder; EF: *Enterococcus faecalis*; SM: *Streptococcus mutans*; PE: *Pseudomonas aeruginosa*; C: control; SC: sterile control; NA: not applicable.

TABLE 1 | DPPH free-radical scavenging activity assay results.

S. no.	Samples	Percentage DPPH free-radical scavenging
1	BTP	52.78%
2	RTP	39.2%
3	KTP	40.9%
4	Ascorbic acid	78.7%

3. Results

3.1. Evaluation of antioxidant activity (DPPH free-radical scavenging assay)

Greater results for DPPH free-radical scavenging were obtained in BTP with 52.78%, followed by KTP (40.9%), and RTP (39.2%) (**Table 1**). The control ascorbic acid showed scavenging activity at 78.7%.

3.2. Determination of *in vitro* antibacterial activity

Based on the results obtained from the *in vitro* antibacterial assay, RTP (Red tooth powder) showed the maximum rate of inhibition of all three bacterial strains when compared to the other two tooth powders. RTP showed promising antibacterial activity with an average zone of inhibition (ZOI) of 20 mm at 10 mg concentration, 16 mm at 5 mg concentration, and 12, 11, and 10 mm for 2.5 mg concentration for *Enterococcus faecalis* (EF), *Streptococcus mutans* (SM), and *Pseudomonas aeruginosa* (PA), respectively (**Figures 2**, **3**). However, the sample BTP (Brindha tooth powder) had shown a moderate inhibition of all three strains with a ZOI of 18, 15, and 16 mm for 10 mg concentration for EF, SM, and PE, respectively. It was 14, 12, and 13 mm for 5 mg, and 9, 11, and 10 mm for 2.5 mg for EF, SM, and PE, respectively (**Figure 3**). A low rate of inhibition was recorded from Kosali tooth powder (KTP) with 11, 12, and 8 mm for EF, SM, and PE strains, respectively, at 10 mg, whereas, no zone of inhibition was found at 5 and 2.5 mg concentrations (**Figure 3**).

3.3. Determination of antibiofilm activity

Although the samples BTP, RTP, and KTP had shown antimicrobial activity against EF, SM, and PE strains, all three samples did not exhibit an antibiofilm effect against the three bacterial strains under study (**Figure 4**).

4. Discussion

Oro-dental disorders arise as a result of a conglomeration of polymicrobial nature leading to various oral infections like dental caries, periodontal diseases, oro-mucosal diseases, and periapical periodontitis (21). The human oral microbiome consists of both pathogenic and probiotic microbes including archaea, protozoa, fungi, viruses, and bacteria (including anaerobic). Concurrently, the imbalance between pathogenic and probiotic microbes causes severe oral pathogenic diseases. Unenviably, oral pathogens are the culprits in causing infective endocarditis leading to atherosclerosis and coronary heart disease (22). This affects especially the population of low economic status in taking care of their oral

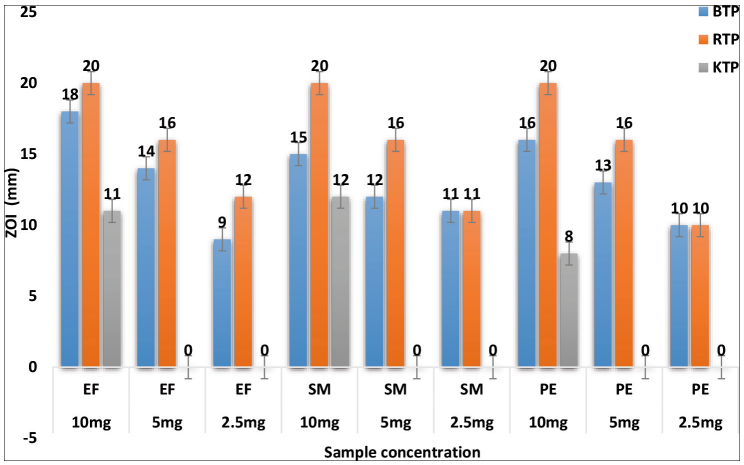

FIGURE 2 | Antibacterial activity assay—zone of inhibition, where BTP: Brindha tooth powder; RTP: Red tooth powder; KTP: Kosali tooth powder; ZOI: zone of inhibition; EF: *Enterococcus faecalis*; SM: *Streptococcus mutans*; PE: *Pseudomonas aeruginosa*.

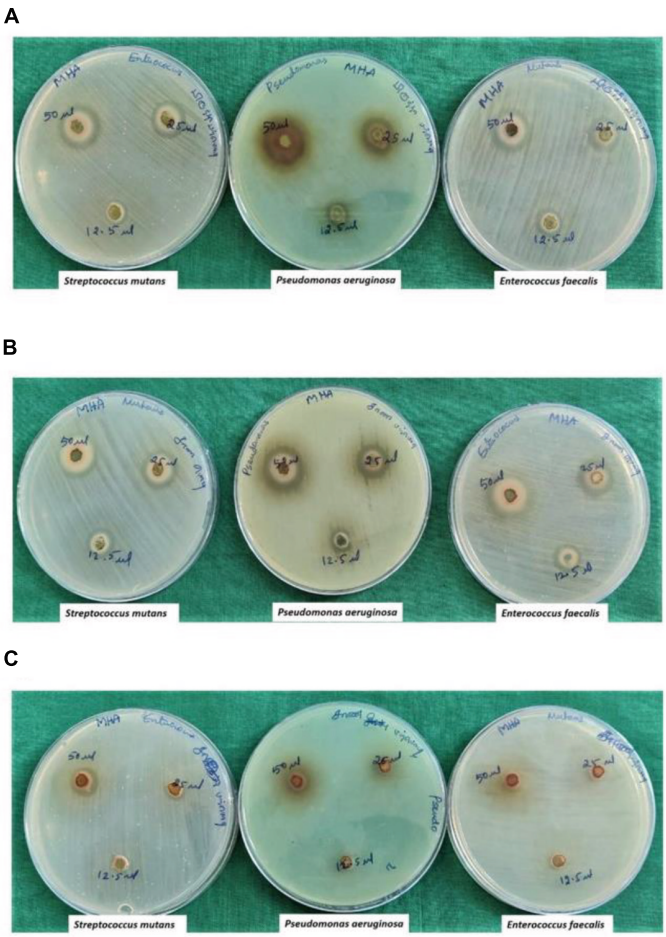

FIGURE 3 | Antimicrobial activity of **(A)** Brindha tooth powder (BTP), **(B)** Red tooth powder (RTP) and **(C)** Kosali tooth powder (KTP) against the test micro-organisms *Streptococcus mutans*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*.

hygiene and medical expenses. In addition, the prolonged use of antibiotics to treat oral pathogens inversely results in the generation of antibiotic-resistant pathogens (12). As a result, the Centers for Disease Control and Prevention (CDC) urges people to practice brushing and flossing their teeth twice a day to prevent dental plaque because "prevention is better than cure." (23).

Recently, natural products have been given increased attention as potential preventative measures for oral disorders. Due to the adverse consequences of the usage of

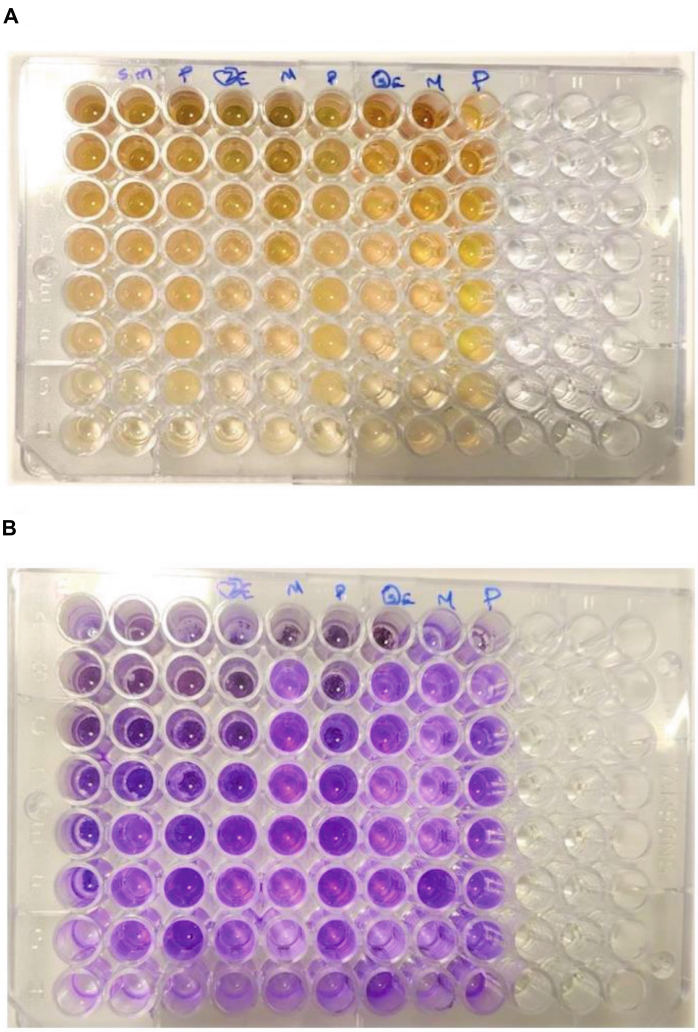

FIGURE 4 | Antibiofilm assay showing the **(A)** microtitre plate after 48 h of incubation and **(B)** crystal violet assay with no biofilm activity for the three toothpastes at varying concentrations.

some dangerous chemicals in the majority of toothpastes and powders on the market, mainly plaque-related disorders such as dental caries have produced an alarming scenario, especially among young children. Herbal toothpaste is prepared from natural products and effectively controls dental plaque and gingivitis which is equivalent to non-herbal and fluoride toothpaste (17, 24). In this study, three different tooth powder formulations, Brindha tooth powder (BTP), Red tooth powder (RTP), and Kosali tooth powder (KTP), were tested for their efficacy against three common pathogenic bacteria *Enterococcus faecalis* (EF), *Streptococcus mutans* (SM), *Pseudomonas aeruginosa* (PE) isolated from the oral cavity of dental patients at Saveetha Dental College and Hospitals (SDC), SIMATS, Chennai, India.

It is a known fact that the progression of leukocytes and free-radical generation has resulted in periodontal diseases. Hence, antioxidants in toothpaste must be able to encounter the plaque-causing pathogenic bacteria and scavenge free radicals. Interestingly, in a recent study, no antioxidant activity was recorded in non-herbal fluorinated toothpaste (25). However, herbal toothpaste has innate antioxidant activity based on the secondary metabolites of medicinal plants (26, 27). In the present study, the average percentage of DPPH free-radical scavenging activity was 52.78, 40.9, and 39.2% for BTP, RTP, and KTP, respectively. In a similar study on the herbal formulation (Dabur Red toothpaste), the activity of scavenging free radicals was 78%, and no activity was found in non-herbal fluorinated toothpaste.

In the current investigation, the results obtained from *in vitro* antimicrobial activity assay (ZOI) indicate that RTP's inhibition activity is the greatest of all three bacterial strains [*Enterococcus faecalis* (EF), *Streptococcus mutans* (SM), and *Pseudomonas aeruginosa* (PE)]. Similarly, both RTP and BTP inhibited the proliferation of all three bacterial strains at high and low concentrations, but KTP failed to inhibit proliferation at lower concentrations.

In this line, the Unani polyherbal toothpaste "Sunoon Zard" inhibited *Staphylococcus aureus* (15.33 mm), *Streptococcus mutans* (17.66 mm), *Streptococcus pyogenes* (14.66 mm), *Streptococcus viridans* (17.33 mm), *Streptococcus*

epidermidis (14.33 mm), *Corynebacterium xerosis* (18.33 mm), *Bacillus cereus* (14.66 mm), *E. coli* (14.33 mm), *Klebsiella pneumoniae* (20.33 mm), *Pseudomonas aeruginosa* (22.33 mm), and *Proteus vulgaris* (13.33 mm) (28). In co-relation with these studies, the present investigation has shown that RTP and BTP inhibit *Enterococcus faecalis*, *Streptococcus mutans*, and *Pseudomonas aeruginosa* at 20 mm/10 mg for RTP, and 18, 15, and 16 mm/10 mg for BTP, respectively, whereas, less activity was recorded for KTP at 11, 12, and 8 mm/10 mg. In another study, among nine different herbal toothpaste formulations, a formulation constituting of extract from pomegranate peel and clove oil demonstrated a high inhibition rate of 26 mm, 27 mm, and 25 mm against *Candida albicans*, *Streptococcus mutans*, and *Staphylococcus aureus*, respectively (29). In another study, some commercial toothpaste had shown antimicrobial activity with the zone of inhibition 25.4, 23, and 22.6 mm for Neem, Colgate, and Meswak toothpaste, respectively, with inhibitory activity against *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans* (30). An interesting study was carried out in Brazil on the antimicrobial activity of toothpaste with natural extracts, chlorhexidine, and triclosan. Among three different kinds of toothpaste, triclosan toothpaste hampers the proliferation of gram-positive bacteria and yeast at a greater rate, but no such activity was seen against *Pseudomonas aeruginosa*. Natural extract toothpaste inhibited all the bacteria including *P. aeruginosa*, but no activity was seen against *Enterococcus faecalis*, whereas, chlorhexidine toothpaste moderately inhibited all the bacteria but not *P. aeruginosa* (31). Fortunately, in this study, all the tooth powder RTP, BTP, and KTP had shown inhibition over *Enterococcus faecalis* and *Pseudomonas aeruginosa*. Although all tooth powders have antibacterial activity, they do not inhibit the biofilm formation on all the three bacterial strains involved.

5. Conclusion

The antioxidant and antibacterial properties of three different natural herbal tooth powders, Brindha tooth powder (BTP), Red tooth powder (RTP), and Kosali tooth powder (KTP), were examined. All of the tooth powder samples displayed moderate levels of free-radical scavenging, with percentages of 52.78, 40.9, and 39.2, respectively. At three different concentrations, RTP effectively inhibited the pathogenic microorganisms *Enterococcus faecalis*, *Streptococcus mutans*, and *Pseudomonas aeruginosa*. In KTP at lower concentrations, there was no inhibition, whereas it was moderate in BTP. Therefore, although lacking an antibiofilm property, all three tooth powders exhibit antioxidant and antibacterial activity.

Ethical waiver issued from IRB/ethical committee

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Authors contributions

RG: executed the work and drafted the manuscript. AG: conceived, design, executed, drafting, evaluated, and approved the manuscript in its final form. PB and SS: reviewed and approved the manuscript in its entirety. RA and PS: provided the test compounds for the study and granted ultimate approval of the article after review. All authors contributed to the article and approved the submitted version.

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