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Antimicrobial efficacy of toothpastes containing herbal vs. synthetic ingredients against antibiotic-sensitive/-resistant Gram -ve oral bacteria that cause dental caries

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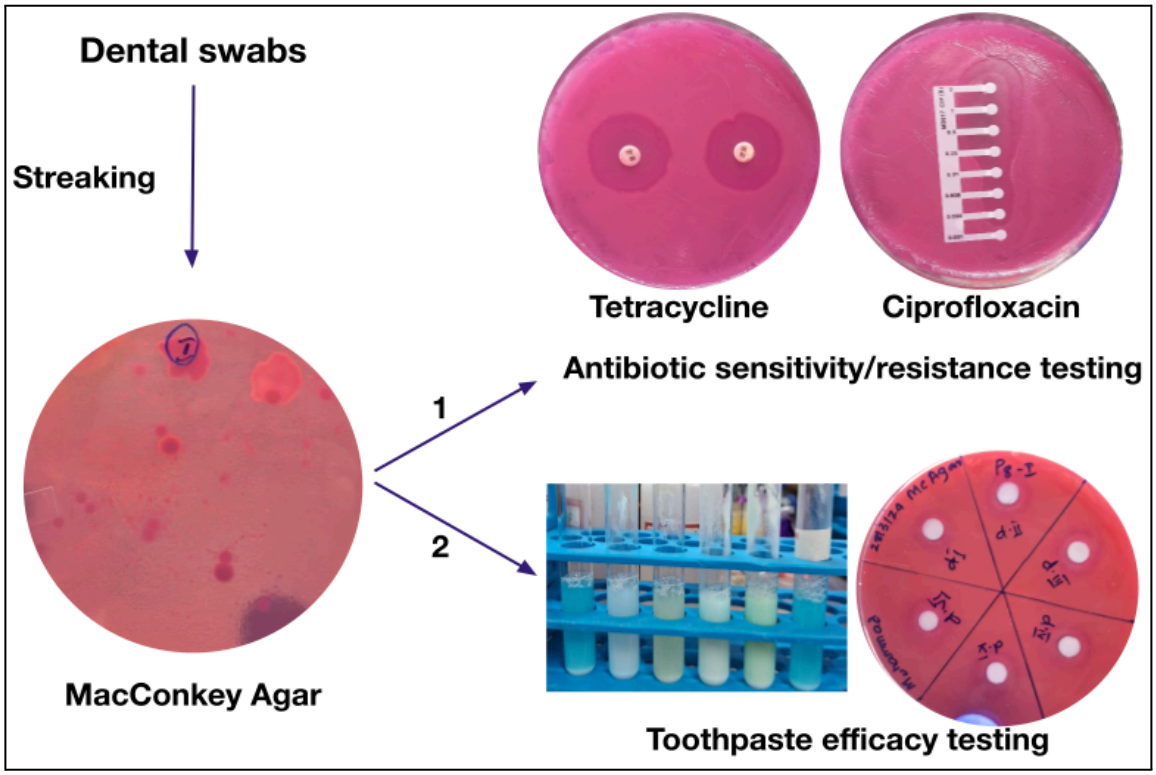
Keywords: Dental caries, toothpaste, antimicrobial efficacy, oral health, antibiotic-resistance, tetracycline, ciprofloxacin.

Maintenance of oral health is very important and it just takes brushing the teeth twice a day in general. Mouthwash is also an alternative approach for brushing. Humans who do not follow these simple rules may end up with dental problems such as cavities, gums related infections, etc. In this study, we obtained 10 dental swabs from patients and evaluated them against six toothpastes that are commonly available in the market in India. Out of the 10 samples that were tested, samples 3, 4 and 8 showed high growth of bacteria on MacConkey agar plates that were found to be tetracycline-sensitive but exhibited differential antibiotic-resistance to ciprofloxacin. Dilutions of all the toothpastes were prepared and the standard well diffusion method was performed. Our results suggest that all 6 toothpastes were able to inhibit samples 4 and 8 but not sample 3. These results have significant implications in understanding not only the oral Gram negative bacteria but also the currently available toothpastes that are effective against them.



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The oral cavity harbors a diverse array of 200 to 300 bacterial species, with only a select few implicated in dental caries or periodontal ailments [1]. Dental caries arise from the demineralization of dental enamel by acidic byproducts from specific bacteria residing in biofilms on teeth, termed dental plaque [2]. Antibiotics are extensively employed in the management of dental caries and other oral health conditions, both for treatment and prevention purposes [3]. Dentists issue approximately 10% of antibiotic prescriptions, varying by country. Furthermore, research indicates that a considerable number of these prescriptions were deemed unnecessary [4]. Regrettably, in recent times, the utilization of antibiotics has been accompanied by the rapid escalation of antibiotic-resistance [5]. Dental caries and periodontal diseases have long been recognized as major oral health challenges in both developed and developing nations, impacting approximately 20–50% of the global population and representing a primary cause of tooth loss [6]. Numerous studies have highlighted the prevalent issue of irrational and excessive antibiotic prescribing for dental conditions, contributing significantly to antibiotic-resistance [5].

Despite the progress made in oral hygiene techniques and the widespread utilization of toothpastes, gram-negative oral bacteria continue to pose a significant challenge in oral health [7]. The presence of gram-negative bacteria, such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, is linked to periodontal conditions like gingivitis and periodontitis, which have the potential to result in tooth loss and systemic health issues [8, 9]. Nonetheless, the effectiveness of traditional toothpaste compositions in managing gram-negative oral bacteria remains inadequately elucidated. This gap in knowledge underscores the necessity for a targeted inquiry into assessing the efficacy of different toothpaste formulations in combatting and diminishing gram-negative oral bacteria. Grasping the impact of toothpastes on gram-negative oral bacteria is crucial for enhancing oral hygiene methodologies, preventing periodontal diseases, and fostering general oral health and well-being**.**

The fundamental aim of this investigation is to assess the efficacy of selected toothpastes on the microbial composition of the oral cavity, particularly focusing on Gram negative bacteria. In this study, we hypothesize that there is high efficiency of formulated toothpaste against Gram negative oral bacteria. In order to address this hypothesis we evaluated the antimicrobial efficacy of different toothpaste formulations against Gram negative oral bacteria and compared the effectiveness of commercial toothpastes in targeting specific species of Gram negative oral bacteria. Additionally we tested the antibiotic-sensitivity or -resistance of the bacteria against standard antibiotics, tetracycline and ciprofloxacin..

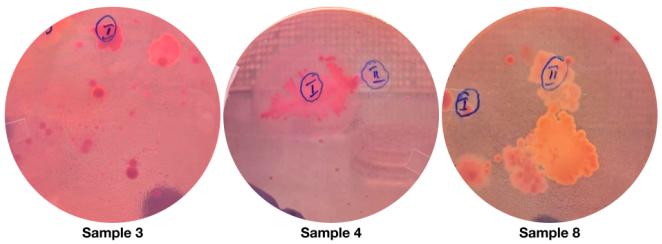

Figure 1. Bacterial colonies obtained from dental swabs.

Materials & Methods:

Collection of dental swabs: Patient dental swabs were obtained from a dental clinic on a research collaboration basis. Dental swabs were obtained by the professionals under the supervision of a physician as a part of their routine check ups in the dental clinic. Each swab was enclosed in a sterile plastic tube with a lid before transportation to the laboratory. The swabs were brought to the laboratory within 1 hour of collection and were used immediately for streaking on MacConkey agar plates.

Media preparation and bacterial culture: MacConkey agar plates were prepared according to the manufacturer's protocol. The media powder was purchased from HiMedia (Cat. No. MH081). According to the manufacturer's instructions, 49.53 g of the powder was dissolved in 1 L of deionized water, stirred and autoclaved for 15 min at 15 lbs pressure and 121 ℃ temperature. Once the media reached lukewarm temperature, it was poured into petri dishes in the laminar airflow chamber under aseptic conditions. The plates with solidified media were then used to streak the dental swabs in the absence of any antibiotics in the plate. All the streaked plates along with a negative control were incubated overnight at 37 ℃.

Antibiotic-sensitivity and -resistance assay: Two standard antibiotics, tetracycline and ciprofloxacin were used in this study. Disk diffusion method was employed to calculate the zones of inhibition. Thirty mcg disks were used for tetracycline and combs containing concentration gradients (0.01 mcg to 2.0 mcg) were used for ciprofloxacin. The dental swabs of samples 3, 4 and 8 were spread on MacConkey agar plates and the antibiotic disks/combs were placed in the plates using sterile forceps. The plates were incubated overnight at 37 ℃. Zones of inhibition were calculated by measuring the diameter of the clear zone from the center of the disk.

Preparation of toothpaste samples: Six toothpastes from different brands including the herbal toothpastes were chosen for this study. Four out of the 6 toothpastes contain herbal composition and two of them contain synthetic formulations.

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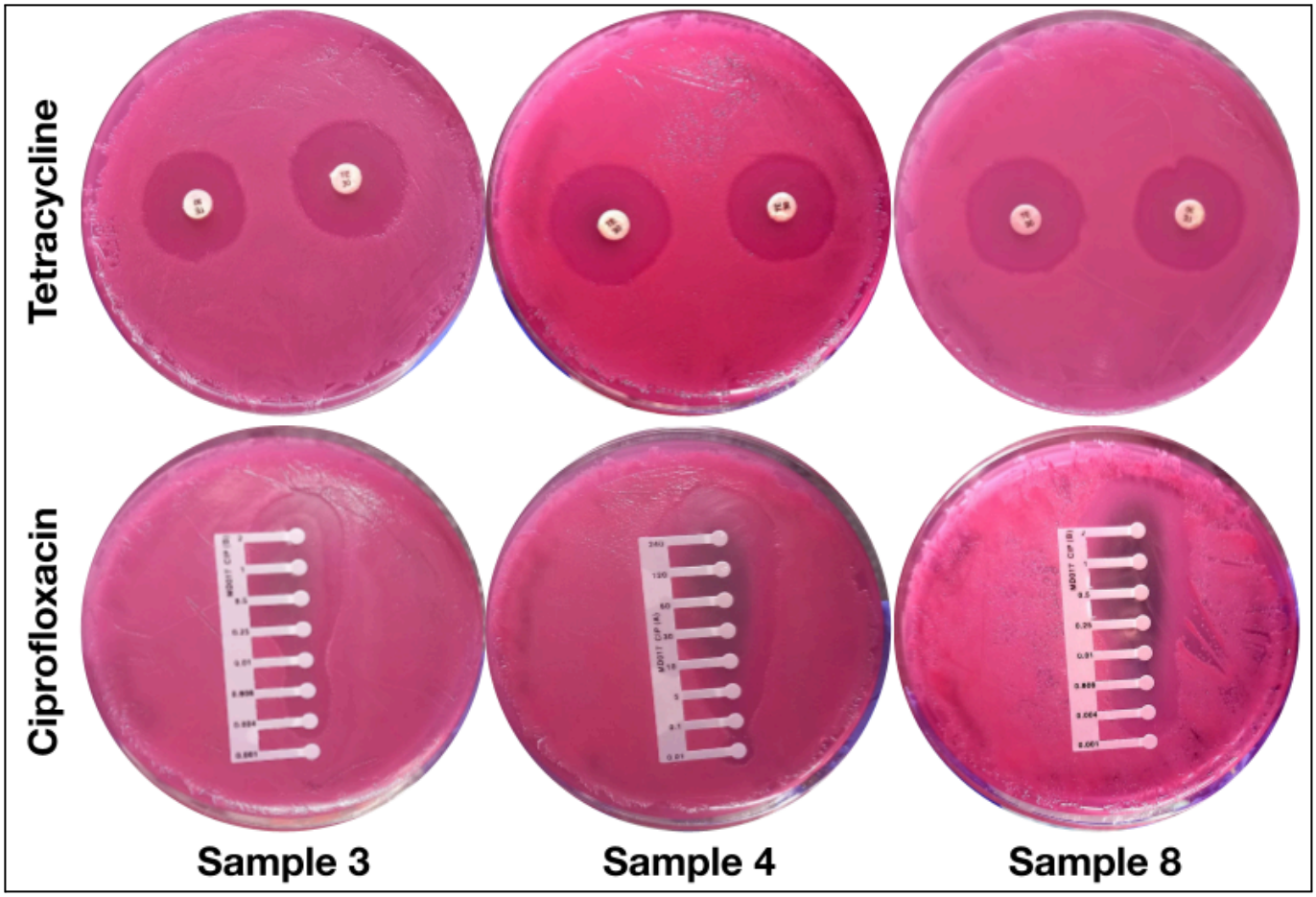

Figure 2. Antibacterial assay for samples 3, 4 and 8.

Evaluation of toothpaste efficacy: Toothpastes were diluted in deionized water in order to dispense them into the wells on the MacConkey agar plates. Dilutions of the toothpastes were made by weighing 1 g of toothpaste into 5 ml of deionized water followed by stirring them well until they dissolved into uniform solutions. The different toothpastes were labeled as I, II, III, IV, V, VI. Well-diffusion method was used to evaluate the toothpaste efficacies. A sterile 8 mm cork-borer was utilized to create six peripheral wells at equal distances in each plate. Each of the six peripheral wells were then filled with 500 µl of the different toothpaste dilutions. The control plate contains the same volume of sterile deionized water, serving as the control. The plates were then transferred to an incubator set at a constant temperature of 37 °C overnight. After the designated incubation time, the antimicrobial efficacy of the dentifrices was assessed by measuring the diameter of the zones of inhibition surrounding each well, a common practice in antimicrobial susceptibility testing. The zones of inhibitions were then compared with each other among the 6 toothpaste samples.

Results and Discussion:

Three out of ten samples yielded high bacterial growth: The total number of dental swabs tested were 10 out of which only 3 yielded a noticeable number of Gram negative bacterial colonies. As shown in Figure 1, samples 3, 4 and 8 displayed colonies. In all three plates, the colonies were categorized as I (pink color lactose fermenters) and II (white color non lactose fermenters). This distinction was to check whether the pathogenicity of colonies from I and II categories is comparable or different. Both I and II category colonies from the samples 3, 4 and 8 were further propagated for antibiotic assay and toothpaste efficacy assay.

Bacteria from the three samples were tetracycline-sensitive but ciprofloxacin-resistant: Antibiotic assays reveal that all samples were tetracycline-sensitive (30 mcg disks) but differentially resistant to ciprofloxacin (gradient combs). As shown in Figure 2, sample 3 displays differential resistance to ciprofloxacin (lack of clear zone of inhibition) compared to the samples 4 and 8 that showed clear zone of inhibition where clear transparency can be seen through the plate.

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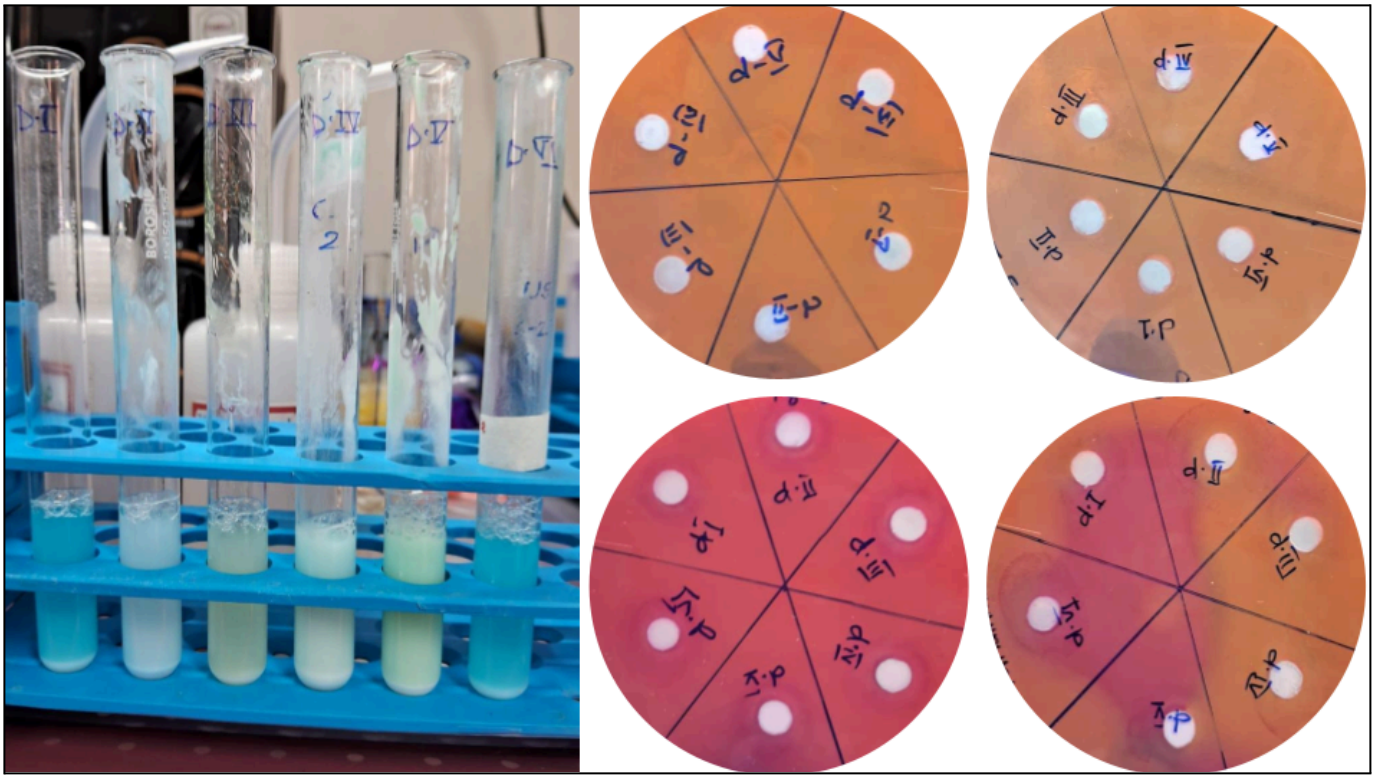

Figure 3. Toothpaste solutions and their efficacy evaluation.

However, a higher concentration gradient comb of ciprofloxacin (0.01 mcg to 240.0 mcg) had to be used for sample 4 due to lack of any visible zone of inhibition. Hence, the zone of inhibition for sample 4 was not compared with samples 3 and 8. The zone of inhibition was calculated with respect to the diameter of the clearance zone. Accordingly, the diameters for the zones of inhibition for tetracycline disks for samples 3 and 8 were measured to be 22.5 $\pm$ 2.12 mm and 23 $\pm$ 0.00 mm, respectively (Figure 2). Although these values are close to each other, the standard deviation of sample 3 was higher compared to sample 8 suggesting that sample 3 may have a combination of both tetracycline- sensitive and -resistance bacteria. Similarly, clear zones of inhibition were obtained for sample 8 with the ciprofloxacin comb but the zones of inhibition for the ciprofloxacin comb in sample 3 were relatively smaller compared to sample 8. Moreover, clear transparency was seen within the zone of inhibition for sample 8 with ciprofloxacin combs which was not the case for sample 3. Taken together, sample 3 can be concluded to contain a mixture of antibiotic-sensitive and -resistant strains of Gram negative bacteria.

All toothpastes were effective against sample 8 but not sample 3: All the 6 toothpastes used in this study showed the zones of inhibition for sample 8 but not for sample 3. As shown in Figure 3, both the lactose fermenters (pink colonies) and non lactose fermenters (white colonies) did not display any visible zones of inhibition for any of the 6 toothpastes used in this study. However, the lactose fermenters (pink colonies) from sample 8 display clear zones of inhibition with equal diameters for all 6 toothpastes. The non lactose fermenters (white colonies) from sample 8 displayed mixed results (Figure 3) which could be due to an overlap of pink and white colonies while plating although evolution of bacterial cells cannot be ruled out. It is noteworthy that both the herbal and synthetic toothpastes showed similar trends in inhibiting the samples 3 and 8 suggesting that the herbal ones were equally potent compared to the synthetic ones that contain triclosan and fluorides.

Conclusion and Future directions:

The current study clearly demonstrates that the oral Gram negative bacteria, irrespective of their antibiotic-sensitivity or -resistance, can pose threat to the existing toothpastes in the market such as the sample 3 tested in this study. However, the antibacterial activity of both herbal and synthetic toothpastes was comparable in inhibiting the oral bacteria as seen in sample 8. Based on these results it is safe to conclude that most of the toothpastes have high efficacy against certain Gram negative oral bacterial species but not against all of the bacteria.

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Conflict of interest: The authors declare no conflict of interest in this study.

Author contributions: M.K. obtained samples from the dental clinic and performed the toothpaste efficacy tests and U.D. performed antibiotic-sensitivity/-resistance assay; M.A. supervised M.K. and U.D.; R.S.Y. is the principal investigator who designed the project, trained M.K., U.D. and M.A., secured required material for the project, provided the laboratory space and facilities needed. R.S.Y. edited and finalized the manuscript.

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Full figure legends:

Figure 1. Bacterial colonies obtained from dental swabs. Out of the 10 dental swabs, samples 3, 4 and 8 shown here yielded more Gram negative bacterial colonies on MacConkey agar plates. Each plate contains both pink colonies (lactose fermenters) designated as "I" and white (non lactose fermenters) designated as "II".

Figure 2. Antibacterial assay for samples 3, 4 and 8. All three samples show clear zones of inhibition with the 30 mcg disks of tetracycline suggesting that they are sensitive to tetracycline. Samples 4 and 8 show clear zones of inhibition with the ciprofloxacin gradient comb but sample 3 displays an opaque zone of inhibition suggesting differential sensitivity/resistance against ciprofloxacin.

Figure 3. Toothpaste solutions and their efficacy evaluation. A total of 6 toothpastes were used to make solutions in deionized water for their efficacy testing. MacConkey agar plates with dental swab sample 3 (top row) and sample 8 (bottom row) were used to perform the well diffusion method. The top and bottom plates on the right side are lactose fermenters while the top and bottom plates on the left side are non lactose fermenters. No zones of inhibition are seen for dental swab sample 3 (top row) but clear zones of inhibition are seen for the dental swab sample 8 (bottom row).