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# ISOLATION OF ORAL PATHOGENS ASSOCIATED WITH PERIODONTAL CONDITIONS AND EVALUATION OF THE ANTIMICROBIAL EFFICACY OF SELECTED COMMERCIAL DENTIFRICES

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**ABSTRACT**

Periodontal disease is a polymicrobial inflammatory condition linked to dental plaque accumulation. Dentifrices are commonly used to aid plaque control and may contain antimicrobial agents; however, their effectiveness varies depending on formulation. This study evaluated microorganisms associated with periodontal conditions and assessed the antimicrobial activity of ten commercially available dentifrices. Fifty oral swab samples were collected and processed using standard microbiological techniques. *Streptococcus mutans*, *Escherichia coli*, and *Candida albicans* were isolated and identified through culture and biochemical tests. The antimicrobial efficacy of ten dentifrices was determined using the Kirby–Bauer disc diffusion method. Zones of inhibition were measured in millimeters and analyzed statistically using one-way ANOVA. A total of 58 isolates were recovered, comprising *S. mutans* (48.28%), *C. albicans* (31.03%), and *E. coli* (20.69%). Antimicrobial activity varied among formulations. Dentifrice B showed the highest antibacterial activity against *S. mutans* (23.6 mm) and *E. coli* (24.8 mm), while dentifrice J demonstrated the strongest antifungal activity against *C. albicans* (23.4 mm). Some formulations exhibited no detectable antimicrobial effect. Commercial dentifrices differ significantly in antimicrobial efficacy. Fluoride-containing formulations demonstrated superior antibacterial activity. Further in vivo studies are required to confirm their clinical effectiveness.

**Keywords**: Periodontal disease; Oral pathogens, Dentifrices; Antimicrobial efficacy; *Streptococcus* mutans

## INTRODUCTION

Periodontal disease represents a group of chronic inflammatory conditions affecting the supporting structures of the teeth, collectively known as the periodontium, which includes the gingiva, periodontal ligament, cementum, and alveolar bone. These diseases range from mild gingivitis, characterized by reversible inflammation of the gingiva, to severe periodontitis, which involves progressive destruction of connective tissue attachment and alveolar bone, potentially leading to tooth mobility and eventual tooth loss (National Institute of Dental and Craniofacial Research, 2013). Periodontal disease remains one of the most prevalent oral health problems worldwide and constitutes a significant public health concern due to its high morbidity and impact on quality of life.

The etiology of periodontal disease is primarily polymicrobial. It is associated with a complex biofilm composed predominantly of Gram-negative, anaerobic, and capnophilic bacteria that colonize the subgingival environment (Skucaite *et al.*, 2010; Ardila and Bedoya-Garcia, 2020). The accumulation of dental plaque a structured microbial community embedded in an extracellular matrix plays a central role in disease initiation and progression. Inadequate oral hygiene practices, particularly ineffective brushing and flossing, promote plaque accumulation and maturation, which subsequently triggers host inflammatory responses that contribute to tissue destruction. Among the microorganisms implicated in periodontal infections are *Streptococcus* species, spirochetes, Bacteroides species, and other opportunistic pathogens (Manupati, 2011).

Several risk factors predispose individuals to periodontal disease, including tobacco smoking, systemic conditions such as diabetes mellitus, hormonal changes during pregnancy, certain medications (e.g., steroids, antiepileptics, and chemotherapeutic agents), poorly fitted dental prostheses, dental crowding, and inadequate oral hygiene (Vargas *et al.*, 2015). These factors may influence both microbial colonization and host immune responses, thereby exacerbating periodontal tissue breakdown.

The human oral cavity harbors a diverse and dynamic microbiota composed of both commensal and potentially pathogenic microorganisms. While commensal bacteria contribute to the maintenance of oral ecological balance, disruption of this balance (dysbiosis) may result in the overgrowth of pathogenic species, leading to oral diseases such as dental caries and periodontal disease (De Oliveira *et al.*, 2020). Additionally, opportunistic fungi such as *Candida albicans* may colonize the oral cavity and contribute to oral infections, particularly under conditions of immune compromise or ecological imbalance.

Mechanical plaque control through tooth brushing and flossing remains the cornerstone of periodontal disease prevention. Dentifrices (toothpastes), used in conjunction with toothbrushes, enhance mechanical plaque removal and may provide additional therapeutic benefits through incorporated active agents. Many commercial dentifrices contain antimicrobial and remineralizing agents such as fluoride compounds, sodium monofluorophosphate, sodium lauryl sulfate, herbal extracts, phosphates, and other bioactive substances (Oyewale, 2005). Fluoride, in particular, is widely recognized for its role in caries prevention and enamel remineralization. However, certain chemical constituents in dentifrices and mouth rinses may be associated with adverse effects, including tooth staining, taste alteration, mucosal irritation, and hypersensitivity reactions (Chang *et al.*, 2001; Beaudouin, 2004).

Given the increasing number of commercially available dentifrices with varied formulations and antimicrobial claims, there is a need for scientific evaluation of their effectiveness against microorganisms associated with periodontal disease.

Understanding the antimicrobial potential of these products is essential for guiding consumer choices and supporting evidence-based oral health practices. Therefore, the present study aimed to isolate pathogenic microorganisms associated with periodontal disease and to evaluate the antimicrobial sensitivity of ten different commercially available dentifrices against selected oral pathogens.

## MATERIALS AND METHODS

### Study Design

The study was conducted to isolate selected pathogenic microorganisms from the oral cavity and evaluate the antimicrobial efficacy of ten commercially available dentifrices.

### Sample Collection

Oral swab samples were collected from consenting individuals after explaining the purpose and procedure of the study. Using sterile cotton swab sticks, the oral cavity 50 individuals was gently swabbed for approximately 15 sec, ensuring adequate contact with the gingival and buccal mucosal surfaces (Sauvik *et al.,* 2013). Each swab was immediately labeled appropriately and transported in a sterile, sealed container to the Microbiology laboratory, Department of Microbiology, Bayero University Kano for processing/analysis.

### Isolation and Identification of Microorganisms

The samples were processed under aseptic conditions. The swabs were inoculated onto appropriate culture media for the isolation of target microorganisms.

For the isolation of *Streptococcus mutans*, specimens were inoculated onto nutrient agar supplemented with 5% sheep blood (blood agar). Chocolate agar was prepared by heating blood agar base to approximately 56°C before the addition of sterile defibrinated sheep blood. The inoculated plates were incubated at 37°C for 24 h in a candle jar to provide a microaerophilic atmosphere. Presumptive *S. mutans* colonies were identified based on colony morphology and Gram staining characteristics, followed by biochemical tests including catalase and coagulase tests as described by Cheesbrough (2004).

For the isolation of *Escherichia coli*, samples were streaked onto MacConkey agar plates and incubated aerobically at 37°C for 24 h. Lactose-fermenting colonies suggestive of *E. coli* were further confirmed using standard biochemical tests including indole production, citrate utilization, and urease tests (Cheesbrough, 2004).

For the isolation of *Candida albicans*, specimens were inoculated onto Sabouraud's Dextrose Agar (SDA) and incubated at room temperature for 48 h. Yeast-like colonies were subjected to microscopic examination and confirmed using the germ tube test according to standard procedures (Cheesbrough, 2004).

### Preparation of Dentifrice Samples

Ten different commercially available dentifrices were obtained from retail outlets. Each product was labeled alphabetically (A–J) to minimize bias during testing. Sterile distilled water was used to prepare dentifrice suspensions where necessary to facilitate diffusion during antimicrobial testing.

### Antimicrobial Susceptibility Testing

The antimicrobial activity of the dentifrices against the isolated microorganisms was evaluated using the Kirby–Bauer disc diffusion method. Pure colonies of each test organism were emulsified in sterile distilled water, and the turbidity of the inoculum was adjusted to match the 0.5 McFarland standard, corresponsding to approximately $1.5 \times 10^8$ CFU/mL.

Sterile Mueller–Hinton agar plates were prepared for bacterial isolates (*S. mutans* and *E. coli*), while Sabouraud's Dextrose Agar plates were used for *C. albicans*. The standardized inoculum (100 µL) was evenly spread across the entire agar surface using a sterile spreader to obtain a uniform lawn culture. The plates were allowed to dry at room temperature before the application of discs.

Sterile 6 mm filter paper discs were impregnated with approximately 50 mg of each dentifrice formulation and aseptically placed on the inoculated agar surface. A sterile blank disc without dentifrice served as the negative control. Each test was performed in triplicate to ensure reproducibility.

The plates inoculated with bacterial isolates were incubated at 37°C for 24 h, while plates inoculated with *C. albicans* were incubated for 48 h. After incubation, the diameter of the zones of inhibition was measured in millimeters using a calibrated ruler. Results were interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS, 2002) guidelines where applicable.

### Preparation of Turbidity Standard

Barium Sulphate (l% w/v) standard suspension was used as turbidity standard. This was prepared by adding 0.6 mL of (l% w/v) Barium Chloride solution with 99.4 mL of $H_2SO_4$ (l% v/v) solution to yield 1% w/v Barium Sulphate suspension. The turbid solution formed was transferred into the test tube as the standard for comparison (Cheesbourgh, 2005).

### Standardization of Inoculum

Using inoculation loop, enough material from an overnight culture of the test organism was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National Committee for Clinical Laboratory Standard (NCCLS, 2008).

### Preparation of Sensitivity Discs

Whatman No. l filter paper discs of 6mm in diameter were punched out with the aid of paper punch and placed in Bijour bottles. They were then sterilized by autoclaving at 121°C for 15 minutes. The discs were allowed to cool.

### Disc Diffusion Test

Standard Inocula of the isolate were swabbed onto the surface of prepared and solidified Mueller Hinton agar in separate Petri-dishes. The prepared discs of the extracts and the standard antibiotic discs (Chloramphenicol) were placed onto the surface of the inoculated media at intervals. The plates were incubated at 37°C for 24 h before observation for and measurement of zones of inhibition (NCCLS, 2008).

### Minimum Inhibitory Concentration (MIC)

MIC was determined by preparing various concentrations of the extracts by serial doubling dilution (as explained in disc preparation) and incorporated into test tubes containing 2mL nutrient broth. Standardized inocula (0.1mL) of the isolates were introduced and the tubes were incubated at 37°C for 24 h (NCCLS, 2008). The results were taken by considering the zone of growth and inhibition of the organisms by the test fraction (Ridgway, 1989).

**Statistical Analysis**

The frequency and percentage distribution of isolated microorganisms were calculated. The mean zone of inhibition for each dentifrice against the test organisms was determined from replicate measurements. One-way analysis of variance (ANOVA) was performed using SigmaPlot statistical software to assess significant differences among dentifrice formulations. A p-value of less than 0.05 was considered statistically significant.

**Ethical Approval**

Ethical approval was obtained from Kano State Ministry of Health with Number: NHREC/17/03/2018.

**RESULTS AND DISCUSSION**

A total of 50 oral swab samples were analyzed in this study. Microbiological examination resulted in the isolation of 58 microbial strains comprising 40 bacterial isolates and 18 fungal isolates. The bacterial isolates included *Streptococcus mutans* (28 isolates; 48.28%) and *Escherichia coli* (12 isolates; 20.69%), while *Candida albicans* accounted for 18 isolates (31.03%) (Table 1). *S. mutans* demonstrated the highest frequency among the recovered microorganisms

**Table 1: Distribution of Isolates and their Percentages**

| Organisms | Number of Isolates | Percentage (%) |
|---|---|---|
| *S. mutans* | 28 | 48.28 |
| *E. coli* | 12 | 20.69 |
| *C. albicans* | 18 | 31.03 |
| Total | 58 | 100 |

The antimicrobial activities of the ten dentifrice formulations against the test organisms were evaluated using the disc diffusion method.

**Antimicrobial Activity against *Streptococcus Mutans***

The dentifrice designated as formulation B exhibited the highest mean zone of inhibition (23.6 mm) against *S. mutans*, followed closely by formulations H (22.6 mm) and A (22.2 mm). Formulations E, F, and G showed no observable zones of inhibition. Formulation J demonstrated the least inhibitory activity among the active dentifrices, with a mean zone diameter of 12.6 mm.

**Antimicrobial Activity against *Escherichia Coli***

Similarly, formulation B demonstrated the highest antimicrobial activity against *E. coli*, with a mean inhibition zone of 24.8 mm. Formulation C also showed strong activity (24.2 mm), followed by formulation H (23.0 mm). Formulations E, F, and G produced no inhibitory effect. Formulation J exhibited the lowest mean inhibition zone (11.4 mm) among the active products.

**Antimicrobial Activity against *Candida Albicans***

The antifungal activity of the dentifrices varied notably. Formulation J exhibited the highest mean inhibition zone (23.4 mm) against *C. albicans*. Formulations C (19.2 mm), D (18.6 mm), and B (18.0 mm) showed moderate antifungal activity. Formulations F and I showed no antifungal effect. Compared with bacterial isolates, a broader range of dentifrices demonstrated measurable inhibitory effects against *C. albicans*.

The antimicrobial efficacy of the dentifrices differed significantly across microbial species and formulations, suggesting that variations in active ingredients and their concentrations influence antimicrobial performance.

**Discussion**

Periodontal disease is a multifactorial and polymicrobial condition strongly associated with the accumulation of pathogenic microorganisms within dental plaque biofilms. In the present study, *Streptococcus mutans* was the most frequently isolated organism (48.28%), followed by *Candida albicans* (31.03%) and *Escherichia coli* (20.69%) (Table 1). The predominance of *S. mutans* corroborates previous reports identifying it as a major colonizer of the oral cavity and a significant contributor to dental caries and biofilm formation (Feroz *et al.*, 2013; Van Winkelhoff and Boutaga, 2005).

The relatively lower isolation rate of *E. coli* may be attributed to the dominance of Gram-positive organisms in the oral microbiota. However, the presence of *E. coli* in oral samples aligns with reports indicating its possible involvement in aggressive and chronic periodontal infections (Asmara *et al.*, 2010). The isolation of *C. albicans* further supports the concept that fungal organisms may contribute to oral dysbiosis, particularly when host defense mechanisms are compromised (Maripandi *et al.*, 2011).

The antimicrobial evaluation of the dentifrices revealed considerable variation in efficacy. Formulation B consistently demonstrated the highest antibacterial activity against both *S. mutans* and *E. coli*. This enhanced activity may be attributed to its fluoride concentration (1450 ppm) and the presence of activated charcoal. Fluoride has long been established as an effective agent in reducing dental caries by enhancing remineralization and inhibiting bacterial metabolism (Harper *et al.*, 1995). The concentration-dependent effectiveness of fluoride-containing products has also been emphasized in previous studies (Fejerskov and Kidd, 2003).

Formulations A and C also exhibited substantial antibacterial activity, likely due to the presence of sodium monofluorophosphate and sodium lauryl sulfate, which possess antimicrobial and surfactant properties. In contrast, formulations E, F, and G demonstrated no inhibitory activity against bacterial isolates. This lack of efficacy may be related to lower concentrations of active antimicrobial agents or the absence of synergistic compounds necessary for effective microbial inhibition (Kamal *et al.*, 2010).

Interestingly, the antifungal activity pattern differed from that observed for bacteria. Formulation J exhibited the highest antifungal activity against *C. albicans*, suggesting that its formulation may contain components with stronger antifungal properties. The variability in antifungal responses supports the notion that antimicrobial efficacy is formulation-dependent and may vary according to microbial structure and physiology.

Herbal-based dentifrices, such as formulation H, demonstrated appreciable antimicrobial effects comparable to some fluoridated products. This finding aligns with reports that herbal extracts such as neem and clove possess natural antimicrobial and anti-inflammatory properties (Amrutesh *et al.*, 2010). The growing interest in herbal formulations

reflects the demand for alternatives with fewer reported adverse effects.

The present findings are consistent with earlier studies indicating that not all commercially available dentifrices exhibit significant antimicrobial activity (Ozaki *et al.*, 2006). The variation observed in this study underscores the importance of evidence-based evaluation of dentifrice formulations rather than reliance solely on marketing claims.

It is important to note that this study was conducted in vitro using the disc diffusion method. While this technique provides valuable preliminary information regarding antimicrobial activity, it does not fully replicate the complex conditions of the oral cavity, where factors such as saliva flow, biofilm architecture, and host immune responses influence antimicrobial effectiveness. Therefore, in vivo studies are recommended to validate these findings under clinical conditions.

## CONCLUSION

This study isolated *Streptococcus mutans*, *Escherichia coli*, and *Candida albicans* from oral samples, with *S. mutans* being the most prevalent organism. The antimicrobial efficacy of the ten tested dentifrices varied considerably. Formulation B showed the highest antibacterial activity, while formulation J demonstrated the strongest antifungal effect. Some dentifrices exhibited little or no antimicrobial activity. These findings indicate that dentifrice effectiveness depends largely on their active ingredients and formulation.

## RECOMMENDATIONS

Dentifrices containing scientifically proven antimicrobial ingredients, particularly those with appropriate fluoride concentrations, should be preferred for effective oral hygiene. Regular tooth brushing at least twice daily is strongly recommended to reduce oral microbial load and prevent periodontal disease. Furthermore, additional clinical studies are necessary to confirm the in vivo effectiveness and long-term benefits of commercially available dentifrices.

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