



Comparison Between the Antimicrobial Efficacy of Herbal and Conventional Toothpaste for Caries Prevention.

Padmalochini Sudharsan

Undergraduate student, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai - 600077

Lavanya Govindaraju,

Associate Professor, Department of Paediatric and preventive dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai- 600077

Corresponding Author:

Padmalochini Sudharsan, Undergraduate student, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai - 600077

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Techniques.

ABSTRACT:

Background:

Dental caries is one of the most common oral diseases worldwide and is primarily associated with cariogenic bacteria such as *Streptococcus mutans*. Toothpastes play a crucial role in maintaining oral hygiene by reducing bacterial load and preventing plaque formation. Conventional toothpastes contain chemical antimicrobial agents, while herbal toothpastes use plant-derived phytochemicals with potential antibacterial properties.

Aim:

The present study aimed to compare the antimicrobial efficacy of commercially available conventional and herbal toothpastes against *Streptococcus mutans*.

Materials and Methods:

An in-vitro study was conducted using agar well diffusion assay to evaluate antibacterial activity. Six different commercially available toothpastes (five conventional and one herbal) were tested against *Streptococcus mutans*. The zone of inhibition (mm) was measured to determine antimicrobial effectiveness. The experiment was performed in triplicate. Statistical analysis was carried out using SPSS version 21.0 with the Kruskal–Wallis test followed by Bonferroni post hoc analysis, and a p -value < 0.05 was considered statistically significant.

Results:

All tested toothpastes showed antimicrobial activity against *Streptococcus mutans*. Among the groups, Gopal toothpaste demonstrated the highest mean zone of inhibition (36.67 mm), while the herbal toothpaste Herbodya showed the lowest (26.33 mm). The differences among the groups were statistically significant ($p = 0.007$).

Conclusion:

Within the limitations of this in-vitro study, conventional toothpastes exhibited higher antimicrobial efficacy compared to the herbal toothpaste tested. However, herbal toothpaste also demonstrated measurable antibacterial activity and may serve as a natural alternative for maintaining oral hygiene.



1. Introduction:

Dental caries is the most common infectious dental problem faced by all age group people around the world. It is most commonly caused by a bacteria *Streptococcus mutans*. It is a tooth adherent cariogenic bacteria which could metabolise the sugars producing acid which could demineralize the tooth structure over a period of time. This is due to change in the homeostasis of the oral cavity which helps the organism to thrive better for their survival. That is why dental caries is also called dietary microbial disease of the oral cavity. Dental caries is considered as the most prevalent disease in the world which affects 100% adults and more prevalent among the low socio- economic status group. Dental carious tissue usually consists of 4 different histological layers namely necrotic zone, contaminated zone, zone of mineralization and translucent zone. Among them, the necrotic zone has a very high microbial load [1].

Oral prophylaxis is most important in caries prevention and improvement in avoiding recurrence. There are many ways to improve the oral hygiene and resistance of the tooth against oral pathogens. Brushing and flossing are highly recommended for both children and the adult population to prevent caries [2]. Fluoride treatment in any forms such as Gel, sealants etc could be a preventive measure. Most importantly fluoride containing toothpaste is highly advised to be used. Fluoride compounds present in the toothpaste could effectively help in remineralization of the tooth, inhibit the demineralization of the enamel and act as anti-cariogenic material. It is also noted that fluoride above the recommended level could cause harmful effects [3] [4].

Antimicrobial agents have been widely used as a chemotherapeutic agent for caries prevention and to maintain oral hygiene. Toothpaste apart from remineralization also has antimicrobial activity against oral pathogens to prevent caries and plaque. The main ingredients such as the chlorhexidine and triclosan are gold standard against the oral pathogens. The mechanism by which they act is they block the synthesis of fatty acids which inhibits the enoyl- acyl carrier protein reductase enzyme [5]. There are conventional and herbal toothpastes available commercially. Conventional toothpaste has the gold standard agents which have high antimicrobial efficacy. But, some studies have

mentioned that these agents have some side effects. Therefore, herbal toothpaste is used as a replacement. Herbal toothpaste does not contain any harmful chemicals. Instead, they contain only herbal ingredients which are friendly to the oral cavity. The ingredients contain phytochemical agents such as phenols, flavonoids, alkaloids, tannins, quinones etc which have a good anti- microbial efficacy [6] [7].

The aim of the study is to compare the anti- microbial efficacy of commercially available conventional and herbal toothpaste for the prevention of caries.

2. Materials and Methods:

Streptococcus mutans are used in this study, The bacterial strains were incubated in nutrient agar slopes at 4°C in the microbiology lab.

Three different commercially available conventional and herbal toothpastes were used in this study and their anti- microbial efficacy were compared.

2.1 Screening of antibacterial activity (Agar well diffusion assay):

Broth culture of the test organisms is prepared and it is compared with 0.5 Mc farland standards. The lawn culture of the broth culture was performed on the Mueller hinton Agar. The wells were cut using sterile cork borers. Different conventional and herbal toothpastes were added to the wells. The culture plate is incubated at 37°C overnight. Then, the zone of inhibition is measured in mm diameter and the results were tabulated accordingly. The test is done in triplicate to avoid inaccuracy.

2.2 Statistical analysis:

The raw data was entered in Microsoft excel sheet and the statistical analysis was done using SPSS version 21.0 (SPSS. Inc., Chicago III., USA) The data obtained from the antimicrobial assay were analyzed using statistical software to determine the differences in antimicrobial efficacy among the tested toothpaste groups. The zone of inhibition (mm) produced by each toothpaste against cariogenic microorganisms was recorded, and the results were expressed as mean, standard deviation, and median values for each group. To determine whether there was a statistically significant difference between the groups, a non-parametric Kruskal–Wallis test was performed. This test was chosen because it allows comparison of more than two independent groups when the sample size is



small. When a statistically significant difference was observed, pairwise comparisons were performed using the Bonferroni post hoc test to identify which specific toothpaste groups differed from each other. The Bonferroni correction was applied to adjust the significance level and reduce the risk of type I error due to multiple comparisons.

A p-value less than 0.05 ($p < 0.05$) was considered statistically significant. The results were presented in the form of tables and graphs to illustrate the differences in antimicrobial activity among herbal and conventional toothpaste formulations.

3. Results:

Anti-bacterial activity of the different toothpastes were evaluated using agar well diffusion assay against *Streptococcus mutans* (Figure 1). The zone of inhibition is recorded (in mm diameter) and presented in table 1.

The (table 2) presents the descriptive statistics of antimicrobial activity of different toothpaste samples measured by the Zone of Inhibition (ZOE) against cariogenic microorganisms. Each toothpaste group had three samples ($N = 3$) tested under identical laboratory conditions.

- Gopal toothpaste showed the highest mean zone of inhibition (36.67 mm), indicating the strongest antimicrobial activity among the tested groups.
- Colgate (32.67 mm) and Close Up (32.33 mm) demonstrated moderate antimicrobial activity.
- Pepsodent (30.67 mm) and Mydent (30.33 mm) showed slightly lower antimicrobial effectiveness.
- Herbodaya, the herbal toothpaste, exhibited the lowest mean zone of inhibition (26.33 mm).

The standard deviation (0.577) across groups indicates very low variability, suggesting consistent results within each group. The overall mean zone of inhibition was 31.5 mm, representing the average antimicrobial activity across all tested toothpastes. A p-value of 0.007 indicates that the difference between the groups is statistically significant ($p < 0.05$), meaning the antimicrobial

effectiveness varies significantly among the toothpaste brands.

In (figure 2) Gopal toothpaste shows the highest ZOE, indicating the strongest antimicrobial activity.

- Colgate and Close Up show moderate antimicrobial activity.
- Mydent and Pepsodent have slightly lower antimicrobial effects.
- Herbodaya shows the lowest ZOE, indicating comparatively weaker antimicrobial efficacy among the tested groups.

The Box plot (Kruskal–Wallis analysis) graph clearly demonstrates variation in antimicrobial activity between herbal and conventional toothpaste formulations.

In (figure 3) the pairwise comparison plot illustrates the differences in antimicrobial activity among the tested toothpaste groups following Bonferroni post hoc analysis. The graph demonstrates that Gopal toothpaste showed the highest rank, while Herbodaya exhibited the lowest rank. The highlighted connections indicate statistically significant differences between specific groups, particularly between Herbodaya and Gopal.

In (Table 3) Post hoc Bonferroni pairwise comparisons revealed a statistically significant difference between Gopal and Herbodaya toothpaste ($p < 0.05$), indicating higher antimicrobial efficacy of Gopal. No other pairwise comparisons showed statistically significant differences after adjustment for multiple comparisons.

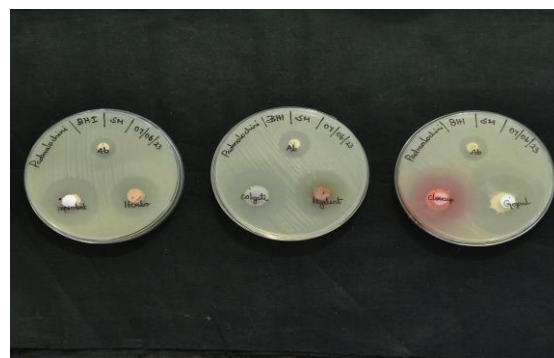


Figure 1: represents the zone of inhibition exhibited by the *Streptococcus mutans* for the conventional and herbal toothpastes.



Table 1: showing the tabulation of the values of the zone of inhibition in mm diameter.

Toothpaste (conventional and herbal)	Zone of inhibition in mm
Close up	32 mm
Gopal	37 mm
Colgate	32 mm
Mydent	30 mm
Pepsodent	31 mm
Herbodaya	26 mm

Herbodaya	3	26.3333	.57735	26.0000	
Total	18	31.5000	3.22217	31.5000	

Table 2: represents the descriptive statistics of antimicrobial activity of different toothpaste samples measured by the Zone of Inhibition (ZOE) against cariogenic microorganisms.

Group	N	Mean	Std. Deviation	Median	p-value
Close up	3	32.3333	.57735	32.0000	0.007
Gopal	3	36.6667	.57735	37.0000	
Colgate	3	32.6667	.57735	33.0000	
Mydent	3	30.3333	.57735	30.0000	
Pepsodent	3	30.6667	.57735	31.0000	
Herbodaya	3	26.3333	.57735	26.0000	

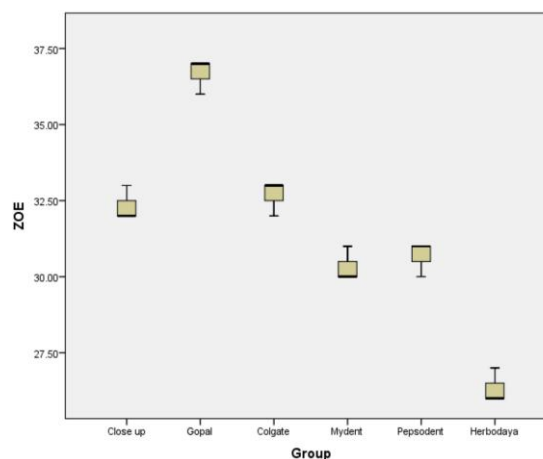


Figure 2: Box plot representing the distribution of zone of inhibition (ZOE) among different toothpaste groups evaluated for antimicrobial activity against cariogenic microorganisms.

Pairwise Comparisons of Group

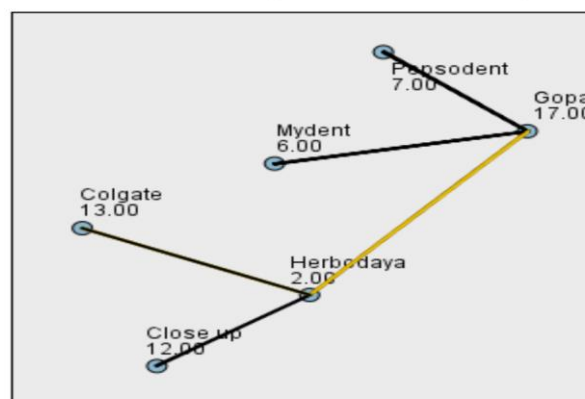


Figure 3: Graphical representation of pairwise comparisons between toothpaste groups using Bonferroni post hoc analysis.



Table 3: represents the Bonferroni post hoc test which determines which specific groups differed significantly after the overall statistical test.

Comparison	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.
Herbodaya – Mydent	4.000	4.318	0.927	0.354	0.354
Herbodaya – Pepsodent	5.000	4.318	1.158	0.247	0.247
Herbodaya – Close up	10.000	4.318	2.316	0.021	0.021
Herbodaya – Colgate	11.000	4.318	2.547	0.011	0.011
Herbodaya – Gopal	15.000	4.318	3.474	0.001	0.001
Mydent – Pepsodent	-1.000	4.318	-0.232	0.817	0.817
Mydent – Close up	6.000	4.318	1.389	0.165	0.165
Mydent – Colgate	7.000	4.318	1.621	0.105	0.105
Mydent – Gopal	11.000	4.318	2.547	0.011	0.011
Pepsodent – Close up	5.000	4.318	1.158	0.247	0.247
Pepsodent – Colgate	6.000	4.318	1.389	0.165	0.165
Pepsodent – Gopal	10.000	4.318	2.316	0.021	0.021
Close up – Colgate	-1.000	4.318	-0.232	0.817	0.817
Close up – Gopal	-5.000	4.318	-1.158	0.247	0.247
Colgate – Gopal	4.000	4.318	0.927	0.354	0.354



4. Discussion:

Dental plaque is the root cause of the dental caries, gingivitis and periodontal diseases. Dental caries are prevalent in all age groups but children are more prone to caries. There are many practical difficulties in maintaining proper oral hygiene in children since cooperation is a determining cause in maintaining oral hygiene. Nowadays in this modern era people fail to maintain proper oral hygiene and their children too. Oral prophylaxis is not a very time consuming process but still people all around the world fail to do so due to various causes. Apart from maintaining proper oral hygiene, food also is a part of caries formation. Sticky food substance consumption should be avoided by the people to avoid caries [8].

Mechanical, chemical and biological methods of oral prophylaxis are available to maintain proper oral hygiene. Among them it is proved that mechanical and chemical methods of cleansing teeth is proved to be the best for maintaining oral hygiene. Brushing with a Toothbrush and paste is a worldwide habit followed by millions of people but this requires a very effective formulation to prevent caries and to maintain oral hygiene in this fast spinning world. [9]. Conventional and herbal toothpaste are available and different people choose varied options. But, there comes a question that conventional or herbal would be oral cavity friendly and which is more effective. Many articles stated that herbal toothpaste had equal or greater effect to conventional toothpaste in caries prevention [6].

Conventional toothpaste contains forms of Fluoride such as sodium fluoride, strontium fluoride and APF which is highly recommended to use a fluoridated toothpaste for all age group people to prevent caries [10]. Apart from fluoride many other compounds such as Triclosan, Abrasives such as silica, aluminium hydroxide and calcium carbonate, Detergents such as sodium lauryl sulphate and cocamidopropyl betaine. Use of these chemicals in the long term may cause some harmful effects. Fluoride on long term use in the soft tissues may lead to necrosis, in hard tissues (teeth) it results in fluorosis [11]. Sodium lauryl sulphate could alter the oral mucosal protein which results in altered blood flow to gingiva. Compared to the beneficial effects of a conventional toothpaste it has many side effects on a long run use. On September 9, 2016 United States FDA

banned incorporation of triclosan in soaps, toothpaste and antiseptics due to its harmful effects. Thereby a famous toothpaste brand which uses triclosan as their main formulation removed and reintroduced their new triclosan free toothpaste [12] [13].

Herbal toothpaste is a good alternative to conventional toothpaste since they do not contain any harsh chemical which is friendly to the oral cavity. Herbal toothpaste contains many herbal ingredients such as neem, clove, babool, amla etc. These herbal products contain phytochemicals such as alkaloids, flavonoids, tannins, phenols, carotenes, etc which are rich in antibacterial activity. They initially prevent plaque accumulation which results in caries prevention. This mechanism of action of caries prevention by herbal toothpaste is highly human friendly, has no any side effects on long term use also. Therefore use of herbal toothpaste is highly recommended. Previous articles also stated that herbal toothpaste has equal effect to conventional toothpaste. It is even good for children under the age of 5 years [14] [15].

From the results of the present study it can be stated that Herbal toothpaste has comparatively less antibacterial activity to conventional toothpaste. But, herbal toothpaste also has a near effect to conventional toothpaste and also has no side effects. Therefore, herbal toothpaste can be suggested for all age group people. Further study has to be performed with increased sample size and spectrophotometer analysis to arrive at a positive result to support the herbal toothpastes.

5. Limitation:

1. Small Sample Size
The study included a limited number of samples (n = 3 per group), which may not fully represent the antimicrobial efficacy of the toothpaste brands.
2. In-vitro Study Design
The antimicrobial activity was evaluated under laboratory conditions using the zone of inhibition method. These conditions may not completely replicate the complex environment of the oral cavity.
3. Limited Microbial Strains
The study focused on cariogenic microorganisms, but dental caries is caused by



a complex microbial biofilm involving multiple bacterial species.

4. **Short-term Assessment**
The antimicrobial activity was measured only at a single time point, without evaluating the long-term effectiveness of the toothpaste formulations.
5. **Variation in Formulations**
Toothpaste brands differ in their ingredients, fluoride concentration, and herbal components, which may influence antimicrobial activity and make direct comparison challenging.

6. Clinical significance:

The findings of this study have important implications for oral health and caries prevention.

- Toothpastes with higher antimicrobial activity may help reduce the growth of cariogenic bacteria, thereby contributing to prevention of dental caries.
- Conventional toothpastes demonstrated greater antimicrobial efficacy compared to the herbal toothpaste tested, suggesting they may provide stronger antibacterial protection.
- However, herbal toothpastes still showed measurable antimicrobial activity and may be preferred by patients seeking natural oral care products.
- Dental professionals can use this information to guide patients in selecting effective toothpaste for maintaining oral hygiene and preventing caries.

7. Future prospects:

Further research is recommended to expand upon the findings of this study.

1. **Larger Sample Size Studies**
Future studies should include more toothpaste brands and larger sample sizes to improve the reliability of results.
2. **In Vivo Clinical Trials**
Clinical studies conducted on human participants would provide more realistic data

regarding the effectiveness of toothpastes in the oral environment.

3. **Evaluation Against Multiple Oral Microorganisms**
Future research could evaluate antimicrobial effects against a broader range of oral pathogens involved in dental caries and periodontal diseases.
4. **Long-term Studies**
Longitudinal studies could assess the sustained antimicrobial effects and caries-preventive potential of herbal and conventional toothpaste.
5. **Analysis of Active Ingredients**
Further studies should investigate the specific active compounds responsible for antimicrobial activity in both herbal and conventional formulations.

8. Conclusion:

Within the limitations of this in-vitro study, the results demonstrated that different toothpaste formulations exhibit varying levels of antimicrobial activity against cariogenic microorganisms.

Among the tested groups, conventional toothpastes showed comparatively higher antimicrobial efficacy, with certain brands demonstrating the largest zones of inhibition. The herbal toothpaste also exhibited antimicrobial properties, although its activity was relatively lower compared to some conventional formulations. These findings suggest that while herbal toothpastes may provide natural alternatives for oral hygiene, conventional toothpastes may offer stronger antibacterial action for caries prevention. Nevertheless, further clinical studies are required to confirm these findings under real oral conditions.

9. AUTHOR CONTRIBUTIONS

Author 1: Padmalochini Sudharsan, carried out the study by collecting data and drafted the manuscript after performing the necessary statistical analysis and in the preparation of the manuscript.

Author 2: Lavanya Govindaraju, aided in conception of the topic, designing the study and supervision of the study, correction and final approval of the manuscript.



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11. CONFLICTS OF INTEREST

None declared

12. FUNDING

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