

Comparative Evaluation Of The Effect Of Chlorhexidine, Alum, Hi-Ora And Terminalia Chebula Extract Mouthwashes On Salivary Microbial Count On 8-13 Year Old Children: A Randomized Clinical Trial

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ABSTRACT

Context: The most common dental diseases are plaque-related infections. In recent years, there has been a lot of concern about caries prevention, especially in the developing countries and lower socio-economic societies. Therefore, there is always a need to find a natural mouthwash that is economical, safe and simple to make in order to improve dental health and be used as a common home treatment. **Aim:** The aim of the present study was to compare the effect of chlorhexidine, alum, hi-ora and *Terminalia chebula* extract mouthwashes on salivary *Streptococcus mutans* and Lactobacilli in 8-13 year old children. **Materials and Methods:** The sample for the study consisted of 60 school children aged 8–13 years. Children were divided randomly into four groups with 15 children each and were asked to rinse with the prescribed mouthwash once daily for 1 month, a gap period of 2 months and again continue the mouthwashes for one month and then discontinue. Simplified Oral Hygiene Index (OHI-S) and Silness – Loe Plaque Index were recorded at baseline, 15th, 30th, 90th, 120th, and 180th day. 2 ml of unstimulated saliva collection was done at baseline, 15th, 30th, 90th, 120th, and 180th day for microbial analysis of *Streptococcus mutans* and *Lactobacillus*. The data's obtained was statistically analyzed using Kruskal Wallis Test and Repeated measures ANOVA, applied and tabulated using the SPSS software version 25.0. **Results:** The results of the study indicate that there was a statistically significant reduction in S. mutans and lactobacilli count in all the four study groups. Terminalia chebula extract mouth rinse was found to be the second most significantly effective antibacterial mouth rinse next to chlorhexidine mouthwash. **Conclusion:** The study demonstrated that alum-containing mouthwash improved plaque inhibition. Herbal mouth rinses such as Hi-ora and Terminalia Chebula have shown promising results, and thus, they can be considered as a newer alternative.

Keywords: Dental caries, mouthwashes, chlorhexidine, alum, hi-ora and *Terminalia chebula*, Lactobacillus, Streptococcus mutans

INTRODUCTION

It has been estimated that the human body is made up of 10^{14} cells of which only 10% are mammalian. The remainders are the microorganisms that make up the resident flora of the host¹. The main pathogen responsible for the initiation of dental caries is thought to be *Streptococcus mutans*^[1]. *Lactobacillus* is more involved with the progression of caries.

Because of the bacterial origin of dental caries, chemotherapeutic agents constitute a reasonable approach towards medical model of management of dental caries. These agents aid in the removal of microorganisms along with the mechanical plaque control, especially in children who are unable, unwilling, or untrained to practice routine effective mechanotherapy^[4].

Traditional mechanical methods has proved to be inadequate for controlling plaque and caries, so endeavor is on latest chemotherapeutic agents for preventing plaque-induced oral diseases. Mouthwash can reach hard-to-clean areas, such as interproximal surfaces, and it can also reduce the growth of biofilms in soft tissues^[2].

Chlorhexidine is considered to be the "gold standard", in mouthwash since it has bactericidal and bacteriostatic effects at low and high doses, respectively^[2]. However, due to its metallic taste and discoloration, it is not advised for usage on a regular basis at home. Because of the mentioned side effects, tremendous research is in progress for an antibacterial agent which is equivalent to chlorhexidine but with less or negligible side effects^[2]. To overcome such side effects, the World Health Organization (WHO) advised researchers to investigate the possible use of natural products such as herbal and natural extracts.

Hi-Ora has an antimicrobial, anti-plaque, antiseptic, analgesic effect and also reduces halitosis and inhibits the growth of cariogenic pathogens. Alum, chemically known as potassium aluminium sulfate, has been traditionally used for its antimicrobial and astringent properties^[5]. *Terminalia chebula*, or Harada, occurs naturally from the sub- Himalayan region of Nepal and northern India. Because of its extraordinary power of healing, this fruit has been termed as "King of Medicine"^[3]. *Terminalia chebula* has anti-bacterial activity against Gram-positive and Gram-negative pathogenic bacteria and also possess anti-fungal and anti-viral properties. The aim of the present study was carried out to compare the effect of chlorhexidine, alum, hi-ora and *Terminalia chebula* extract mouthwashes on salivary *Streptococcus mutans* and lactobacilli in 8-13 year old children.

MATERIALS AND METHODS

STUDY DESIGN :

The present study comprised of 60 healthy children aged between 8 – 13 years, which were selected from Lok Uthhan Welfare Society, Housing Board Colony, Ayodhya Nagar, Bhopal and patients visiting the Department of Pediatric and Preventive Dentistry, People's Dental Academy, Bhopal. The research was initiated after obtaining approval from the Institutional Ethical Committee on 06/05/2022, reference number - IEC/2022/700/02. Written informed consent from caretaker/ parents were obtained prior to the study. The selection of the subjects

were made using following inclusion and exclusion criteria.

INCLUSION CRITERIA:

- Children with age group between 8 -13 years.
- Children who had a minimum of one to two established carious lesions.
- Patients who are willing to participate in the study.
- Patient who can come for follow up.

EXCLUSION CRITERIA:

- History of current or past 1-month antibiotic usage.
- Cellulitis, abscess, draining sinus, or other emergency dental treatment.
- Use of oral hygiene aids other than routine tooth brushing.
- Individuals having systemic diseases.
- Those who are already using any mouth rinses.

The selected children were divided into 4 groups, in each group 15 children were taken.

The group designed were as follows :-

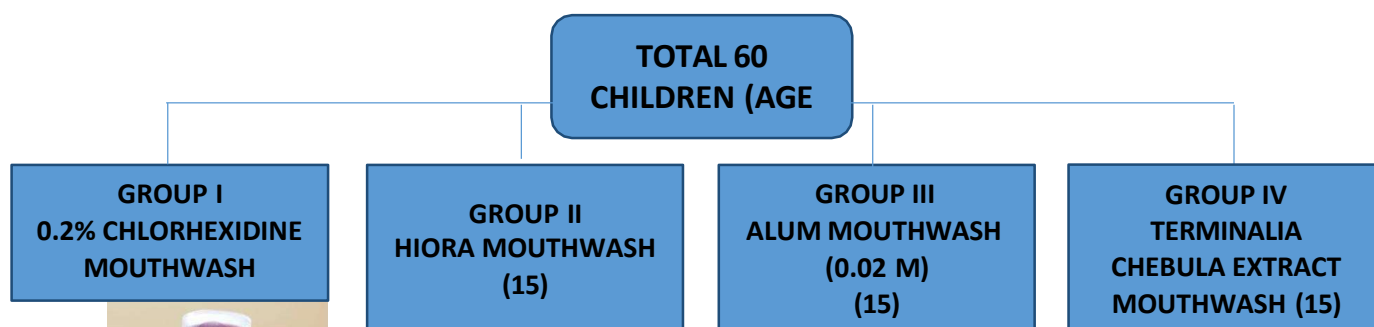


Figure 1 : 0.2% Chlorhexidine Mouthwash



Figure 2 : 0.02 M Alum Mouthwash



Figure 3 : HiOra Mouthwash



Figure 4 : *Terminalia chebula* Extract

PREPARATION OF MOUTHWASHES

a) Method of Preparing 0.02 M Potash Alum Solutions ^{[2],[5]} :-

Alum, 0.02 M (chemically known as potassium aluminum sulfate) mouth rinse was prepared by taking weighed quantity of potassium aluminum sulfate which is found in its dodecahydrate form [molecular formula $KAl(SO_4)_2 \cdot 12H_2O$]. It was calculated using its molecular weight: $474.39 \text{ (wt of alum)} = \text{mol. wt} \times 0.02 = 9.4878 \text{ g}$. Initially, it was dissolved in 800 ml of distilled water and to it, 1 g of sodium benzoate as a preservative and 0.5 g of sodium saccharine (as a sweetening agent) were added.

To 200 ml of distilled water, 0.5 ml of Tween 20 as a emulsifying agent and 0.5 ml of peppermint oil were added and mixed properly. The resultant mixture was mixed with 800 ml of alum solution with the help of a propeller, to formulate a clear mouth rinse.

The pH of the solution was maintained between 3.0 and 3.5 and this can be adjusted by using sodium hydroxide.

All the materials for the preparation of Alum mouth wash was provided by S. K. Traders, Ashoka Enclave, Bhopal.

b) Method of preparation of terminalia chebula mouth wash^[4] :-

The dried ripe fruits of *T. chebula* were obtained and ground to fine powder and 10% concentration of cold aqueous extracts were prepared. Cold aqueous extract was prepared by suspending the ripe fruit (400 gm) in 10 times its quantity of sterile distilled water in a round bottomed flask and kept at 4°C for 72 hours. The aqueous extract was then decanted, clarified by filtration through a muslin cloth, and evaporated in a flat-bottomed porcelain dish at 40°C. The dried extract 'was stored at 4°C prior to use. The extract was suspended in polyethylene glycol (PEG) 400 (20% v/v) and sterile distilled water to give a final concentration of 30% w/v. The concentrated extract was diluted with sterile distilled water to give concentrations of 10% w/v.

Terminalia Chebula extract mouthwash was prepared at Dhathri Life Sciences Private Limited, Kerala.

RINSING PROCEDURE

The children were given the mouthwashes based on the group they belong to. They were instructed to measure 10ml of undiluted mouthwash using a graduated dispensing cup, pour the same into a disposable cup and then rinse by swishing the undiluted solution for 60 seconds once daily after meal. The children were advised not to eat or rinse for the next 30 minutes.

6.SAMPLE COLLECTION

The duration of the study was 6 months, which was divided into four phases.

All the children were given instructions to follow the same oral hygiene practices for 1 month, a gap period of 2 months and again continue the mouthwashes for one month and then discontinue.

Simplified Oral Hygiene Index (OHI-S) and Silness – Loe Plaque Index were recorded at baseline, 15th, 30th, 90th, 120th, and 180th day. 2 ml of unstimulated saliva collection was done at baseline, 15th, 30th, 90th, 120th, and 180th day between 10 and 10.30 AM to match the circadian rhythm for microbial analysis of *Streptococcus mutans* and *Lactobacillus*.

7.MICROBIAL ANALYSIS

Children undergoing investigations were given clear instructions to refrain from eating for 1 h

before collection of saliva.

Two ml of unstimulated saliva was collected by asking the children to let saliva collect in the floor of the mouth without swallowing it for at least 1 min and then to expectorate into the sterile container. The sterile container was then immediately transported to the microbiology laboratory, where it was processed immediately.

Hundred microliters of saliva was diluted with 1 ml of normal saline (1:10 dilution). Using an inoculation loop, 5 of the 1:10 dilution sample was streaked on Mitis Salivarius-Bacitracin (MSB) agar and Rogosa agar (MRS agar) (Hi-Media Laboratories, Mumbai, India), a selective medium for *S. mutans* and *Lactobacilli* species respectively. After incubation for 48 h at 37°C, microbial counting was performed using a digital colony counter and the counts of *S. mutans* and *lactobacilli* was expressed as number of colony forming units/ml.

Microbial analysis was carried out at the Molecular Biotechnology Laboratory, People's University, Bhopal.

STATISTICAL ANALYSIS

Data was entered in Microsoft Excel spreadsheet and data was analyzed using statistical package of social sciences 25.0 software (IBM, Chicago, IL, USA). Analysis of the data was done using Kruskal Wallis Test and Repeated measures ANOVA . For all statistical purposes, a p-value of ≤ 0.05 was considered significant.

RESULTS

Table 1- Comparison of mean OHI-S scores between different time intervals in each study group (n-60)

Groups	Time interval	N	Mean	SD	Min	Max	p-Value
Group I – Chlorhexidine mouthwash	Day 1	15	1.42	0.99	0.86	1.97	0.000*
	Day 15	15	0.60	0.70	0.20	0.99	
	Day 30	15	0.30	0.41	0.06	0.53	
	Day 90	15	0.16	0.33	- 0.01	0.35	
	Day 120	15	0.02	0.05	- 0.01	0.05	
	Day 180	15	0.06	0.18	- 0.04	0.16	
Group II –Alum mouthwash	Day 1	15	1.42	1.21	0.75	2.10	0.000*
	Day 15	15	0.94	1.03	0.37	1.51	
	Day 30	15	0.27	0.38	0.06	0.48	
	Day 90	15	0.14	0.19	0.03	0.24	
	Day 120	15	0.04	0.08	0.00	0.09	
	Day 180	15	0.00	0.02	- 0.00	0.02	

Group III –Hi-ora mouthwash	Day 1	15	1.20	0.89	0.70	1.69	0.000*
	Day 15	15	0.77	0.74	0.36	1.18	
	Day 30	15	0.42	0.55	0.11	0.72	
	Day 90	15	0.26	0.40	0.04	0.49	
	Day 120	15	0.14	0.28	-	0.30	
					0.01		
	Day 180	15	0.02	0.05	-	0.05	
Group IV – Terminalia Chebula Extract mouthwash	Day 1	15	1.16	1.16	0.52	1.81	0.000*
	Day 15	15	0.85	0.91	0.34	1.35	
	Day 30	15	0.53	0.67	0.16	0.90	
	Day 90	15	0.43	0.59	0.10	0.76	
	Day 120	15	0.30	0.45	0.05	0.55	
	Day 180	15	0.33	0.43	0.09	0.57	

SD- Standard Deviation, N= number of subjects,*statistically significant

Table 1 shows comparison of mean OHI-S scores between different time intervals in each study group. Upon comparing the OHI-S (oral hygiene index-simplified) of each group's members at six distinct intervals (days 1, 15, 30, 60, 90, 120, and 180 days), group 1 (chlorhexidine mouthwash) demonstrated a statistically significant difference (p=0.000) between the six intervals. The mean OHI-S score was 1.42 at baseline; however, after 120 days, it was 0.02, and after 180 days, it was 0.06. The mean OHI-S score for group II (alum mouthwash) was 1.42 on day 1, dropped to 0.04 after 120 days and then 0.006 after 180 days. This difference between the six intervals was highly significant statistically. The mean OHI-S score in group III (Hi-ora mouthwash) was 1.20 at baseline, 0.14 after 120 days and 0.02 after 180 days. The difference between the six intervals was highly significant statistically (p=0.000). The mean OHI-S score in group IV (mouthwash containing Terminalia chebula extract) was 1.16 at baseline, 0.30 after 120 days, and 0.33 after 180 days. This difference was statistically significant (p<0.05).

Table 2- Comparison of mean OHI-S score between 4 groups at different intervals (n=60)

Time	Groups	N	Mean Rank	Chi square	p- value
Day 1	I	15	32.97	0.835	0.841
	II	15	31.93		
	III	15	29.23		
	IV	15	27.87		
DAY 15	I	15	27.67	0.565	0.904
	II	15	31.87		
	III	15	31.40		
	IV	15	31.07		
DAY 30	I	15	27.43	1.936	0.586

	II	15	27.67	1.501	0.682
	III	15	34.10		
	IV	15	32.80		
DAY 90	I	15	27.50	5.745	0.125
	II	15	29.43		
	III	15	30.80		
	IV	15	34.27		
DAY 120	I	15	24.87	7.556	0.05 *
	II	15	28.53		
	III	15	31.83		
	IV	15	36.77		
DAY 180	I	15	30.00		
	II	15	26.23		
	III	15	28.13		
	IV	15	37.63		

Table 2 shows comparison of mean OHI-S score between 4 groups at different intervals. When comparing the intergroup OHI-S score, the difference was not statistically significant at days 1, 15, 30, 90, 120 days but 180th day the difference became statistically significant (0.05).

Table 3- Comparison of mean Plaque Index scores between different time intervals in each study group (n=60)

Groups	Time interval	N	Mean	SD	Min	Max	p-Value
Group I – Chlorhexidine mouthwash	Day 1	15	0.92	0.75	0.50	1.34	0.000*
	Day 15	15	0.43	0.64	0.07	0.79	
	Day 30	15	0.22	0.42	-0.01	0.45	
	Day 90	15	0.29	0.37	0.08	0.49	
	Day 120	15	0.06	0.11	0.00	0.13	
	Day 180	15	0.16	0.39	-	0.38	
Group II –Alum mouthwash	Day 1	15	0.83	0.91	0.32	1.34	0.002*
	Day 15	15	0.33	0.49	0.06	0.60	
	Day 30	15	0.16	0.28	0.00	0.32	
	Day 90	15	0.22	0.42	-0.01	0.45	
	Day 120	15	0.05	0.13	-0.02	0.12	
	Day 180	15	0.07	0.18	-0.02	0.17	
Group III –Hi-ora mouthwash	Day 1	15	0.74	0.59	0.41	1.06	0.000*
	Day 15	15	0.26	0.37	0.06	0.47	

	Day 30	15	0.07	0.14	-0.00	0.15	
	Day 90	15	0.18	0.29	0.01	0.34	
	Day 120	15	0.12	0.23	-0.00	0.25	
	Day 180	15	0.20	0.32	0.01	0.38	
Group IV – Terminalia Chebula Extract mouthwash	Day 1	15	0.88	0.88	0.39	1.37	0.001*
	Day 15	15	0.45	0.67	0.08	0.82	
	Day 30	15	0.24	0.44	0.00	0.49	
	Day 90	15	0.22	0.41	-0.00	0.45	
	Day 120	15	0.09	0.26	-0.05	0.23	
	Day 180	15	0.02	0.07	-0.01	0.07	

SD- Standard Deviation, N= number of subjects,*statistically significant

Table 3 shows comparison of mean Plaque Index scores between different time intervals in each study group. When the Plaque score was compared at six different intervals, group 1 (mouthwash containing chlorhexidine) demonstrated a significant difference ($p=0.000$) between the intervals. The mean PI score was 0.92 at baseline; however, after 120 days, it was 0.06 and after 180 days it was 0.16. The mean PI score for group II (alum mouthwash) was 0.83 on day 1, dropped to 0.05 after 120 days, and then to 0.07 after 180 days. The difference between the six intervals was statistically significant ($p=0.002$). The mean PI score in group III (Hi-ora mouthwash) was 0.74 at baseline, dropped to 0.12 after 120 days, and then to 0.20 after 180 days. The difference between the six intervals was statistically significant. The mean PI score for group IV (Terminalia Chebula Extract mouthwash) was 0.88 at baseline, 0.09 after 120 days and 0.02 after 180 days. There was a statistically significant difference between the six intervals ($p=0.001$).

Table 4- Comparison of Mean rank of Plaque index score between 4 groups at different intervals (n=60)

Time	Groups	N	Mean Rank	Chi square	p- value
Day 1	I	15	33.13	0.483	0.92
	II	15	29.17		
	III	15	29.67		
	IV	15	30.03		
DAY 15	I	15	31.70	0.828	0.84
	II	15	27.53		
	III	15	30.07		
	IV	15	32.70		
DAY 30	I	15	30.43	0.609	0.89
	II	15	31.30		
	III	15	28.23		

	IV	15	32.03		
DAY 90	I	15	34.63	1.528	0.67
	II	15	28.73		
	III	15	28.50		
	IV	15	30.13		
DAY 120	I	15	32.20	0.795	0.85
	II	15	28.80		
	III	15	31.83		
	IV	15	29.17		
DAY 180	I	15	32.07	3.563	0.31
	II	15	29.43		
	III	15	34.83		
	IV	15	25.67		

Table 4 shows comparison of Mean rank of Plaque index score between 4 groups at different intervals. At day 1, day 15, day 30, day 90, day 120 and day 180, the difference in the intergroup PI scores was not statistically significant.

Table 5- Comparison of mean CFUs (*S. mutans*) between different time intervals in each study group (n-60)

Groups	Time interval	N	Mean CFUs	SD	Min	Max	p-Value
Group I – Chlorhexidine mouthwash	Day 1	15	41.5413	3.46117	39.625	43.458	0.000*
	Day 15	15	21.4315	2.31442	20.150	22.713	
	Day 30	15	10.9691	1.55407	10.109	11.830	
	Day 90	15	10.8849	1.66876	9.961	11.809	
	Day 120	15	6.4049	1.41062	5.624	7.186	
	Day 180	15	6.0944	1.54573	5.238	6.950	
Group II –Alum mouthwash	Day 1	15	41.8537	2.46867	40.487	43.221	0.000*
	Day 15	15	34.0061	3.72328	31.944	36.068	
	Day 30	15	24.6270	1.94289	23.551	25.703	
	Day 90	15	23.9835	2.22102	22.754	25.213	
	Day 120	15	15.5487	1.57032	14.679	16.418	
	Day 180	15	14.6523	1.96510	13.564	15.741	
Group III –Hi-ora mouthwash	Day 1	15	41.4773	3.98978	39.268	43.687	
	Day 15	15	27.0642	2.04322	25.933	28.196	

	Day 30	15	16.9873	1.53972	16.135	17.840	0.000*
	Day 90	15	16.3881	.96468	15.854	16.922	
	Day 120	15	12.1856	.67168	11.814	12.558	
	Day 180	15	11.6189	.98886	11.071	12.166	
Group IV – Terminalia Cnebulia Extract mouthwash	Day 1	15	41.7961	2.03975	40.667	42.926	0.000**
	Day 15	15	27.6361	2.16348	26.438	28.834	
	Day 30	15	17.4272	1.40839	16.647	18.207	
	Day 90	15	16.3669	1.37548	15.605	17.129	
	Day 120	15	12.0607	1.04526	11.482	12.640	
	Day 180	15	11.3885	.97555	10.848	11.929	

SD- Standard Deviation, N= number of subjects,*statistically significant

Table 5 shows comparison of mean CFUs (*S. mutans*) between different time intervals in each study group. When the streptococcus mutans colony count for each group at different intervals is compared in the current study, the difference between the six intervals is statistically significant ($p=0.001$). In group I, the mean CFU at base line was 41.54, at 120 days it was 6.40, and at 180 days it was 6.09. The mean CFUs of *S. mutans* in group II (alum mouthwash) were 41.54 on day 1, 15.54 after 120 days, and 14.65 after 180 days. The difference between the six intervals was statistically significant ($p=0.000$). The mean CFUs in group III (Hi-ora mouthwash) were 41.47 at baseline, 12.18 after 120 days, and 11.61 after 180 days. This difference was statistically significant ($p=0.000$) between the six intervals. The mean CFUs in group IV (mouthwash containing Terminalia chebula extract) were 41.79 at baseline, 12.06 after 120 days, and 11.38 after 180 days. This difference was statistically significant ($p < 0.05$).

Table 6- Comparison of mean CFUs (*S. mutans*) between 4 groups at different intervals (n=60)

Time	Groups	N	Mean Rank	Chi square	p- value
Day 1	I	15	31.17	0.453	0.92
	II	15	30.50		
	III	15	32.23		
	IV	15	28.10		
DAY 15	I	15	9.07	41.04	0.000*
	II	15	49.67		
	III	15	30.00		
	IV	15	33.27		
DAY 30	I	15	8.00	49.93	0.000*
	II	15	53.00		
	III	15	29.33		
	IV	15	31.67		

DAY 90	I	15	8.13	49.507	0.000*
	II	15	53.00		
	III	15	30.20		
	IV	15	30.67		
DAY 120	I	15	8.00	49.64	0.000*
	II	15	52.93		
	III	15	30.60		
	IV	15	30.47		
DAY 180	I	15	8.00	44.33	0.000*
	II	15	50.27		
	III	15	32.47		
	IV	15	31.27		

Table 6 shows comparison of mean CFUs (*S. mutans*) between 4 groups at different intervals. When we examine the CFUs between the groups, on day one there was no statistically significant difference, but on days 15, 30, 90, 120, and 180, there was a statistically significant difference ($p < 0.05$).

Table 7 - Comparison of mean CFUs (*Lactobacillus*) between different time intervals in each study group (n=60)

Groups	Time interval	N	Mean	SD	Min	Max	p-Value
Group I – Chlorhexidine mouthwash	Day 1	15	21.1213	1.57585	20.249	21.994	0.000*
	Day 15	15	12.7542	1.23881	12.068	13.440	
	Day 30	15	10.1156	0.79681	9.674	10.557	
	Day 90	15	9.6961	0.90180	9.197	10.196	
	Day 120	15	7.1670	0.79995	6.724	7.610	
	Day 180	15	6.5192	1.05454	5.935	7.103	
Group II –Alum mouthwash	Day 1	15	21.4290	1.49369	20.602	22.256	0.000*
	Day 15	15	17.9722	1.64862	17.059	18.885	
	Day 30	15	14.9608	1.59888	14.075	15.846	
	Day 90	15	14.3088	1.46901	13.495	15.122	
	Day 120	15	11.7772	1.20584	11.109	12.445	
	Day 180	15	11.2155	1.18596	10.559	11.872	
Group III –Hi-ora mouthwash	Day 1	15	20.9564	1.51411	20.118	21.795	0.000*
	Day 15	15	16.2480	1.43109	15.455	17.041	
	Day 30	15	12.8185	0.79105	12.380	13.257	

	Day 90	15	11.8058	0.99933	11.252	12.359	
	Day 120	15	9.6273	1.02753	9.058	10.196	
	Day 180	15	9.4598	1.51381	8.621	10.298	
Group IV – Terminalia Chebula Extract mouthwash	Day 1	15	21.4842	1.48764	20.660	22.308	
	Day 15	15	16.5667	1.09025	15.963	17.170	
	Day 30	15	12.5411	1.00349	11.985	13.097	
	Day 90	15	11.6909	1.01818	11.127	12.255	0.000**
	Day 120	15	9.3918	1.07206	8.798	9.985	
	Day 180	15	9.0697	.64056	8.715	9.424	

Table 7 shows comparison of mean CFUs (Lactobacillus) between different time intervals in each study group. When the lactobacillus colony count for each group is compared at different intervals in the current study, the mean CFU in group I was 21.12 at baseline, 7.16 at 120 days, and 6.51 at 180 days. The difference between the six intervals was statistically significant ($p=0.000$). The mean CFUs of Lacto bacillus in group II (alum mouthwash) were 21.42 on day 1, decreased to 11.77 after 120 days, and then to 11.21 after 180 days. This difference was statistically significant ($p=0.000$). The mean CFUs in group III (Hi-ora mouthwash) were 20.95 at baseline, decreased to 9.62 after 120 days, and then to 9.45 after 180 days. This difference was statistically significant ($p=0.000$). The mean CFUs in group IV (mouthwash containing Terminalia chebula extract) were 21.48 at baseline, 9.39 after 120 days, and 9.06 after 180 days. The difference between these six intervals was statistically significant ($p<0.05$).

Table 8- Comparison of mean CFUs (lactobacillus) between 4 groups at different intervals (n-60)

Time	Groups	N	Mean Rank	Chi square	p- value
Day 1	I	15	28.67	1.24	0.74
	II	15	32.73		
	III	15	27.40		
	IV	15	33.20		
DAY 15	I	15	8.47	37.15	0.000
	II	15	46.27		
	III	15	32.67		
	IV	15	34.60		
DAY 30	I	15	8.67	40.45	0.000
	II	15	48.80		
	III	15	33.80		
	IV	15	30.73		
DAY 90	I	15	10.27	40.02	0.000
	II	15	50.57		

	III	15	31.50	43.32	0.000
	IV	15	29.67		
	I	15	8.47		
	II	15	50.27		
DAY 120	III	15	32.67	40.62	0.000
	IV	15	30.60		
	I	15	9.07		
	II	15	49.40		
DAY 180	III	15	33.50	40.62	0.000
	IV	15	30.03		
	I	15	9.07		
	II	15	49.40		

Table 8 shows comparison of mean of CFUs (lactobacillus) between 4 groups at different intervals. When we examine the CFUs between the groups, on day 1 there was no statistically significant difference, but on days 15, 30, 90, 120, and 180, there was a statistically significant difference ($p < 0.05$).

DISCUSSION

The surface of the oral cavity is constantly colonized by microorganisms. One milliliter of whole saliva may contain more than 200 million organisms representing more than 250 different species²². Streptococcus constitutes an essential part of the microflora which constantly colonizes the mucous membrane and the teeth. Mutans streptococci are mainly responsible for the initiation of the caries lesion especially in the enamel^[1].

Lactobacilli have been considered as major contributors to dental caries for over a century. There are various species of lactobacillus in oral cavity and the ability of lactobacilli to form biofilms is poor, although differences exist between the different major species. Lactobacillus species are frequently identified at active carious sites, especially in lesions with advanced caries in adult and paediatric patients.

Chlorhexidine mouthwash is considered to be the “gold standard” due to its bacteriostatic and bactericidal properties at low and high concentrations, respectively^[23]. The findings of the present study are in agreement with the study done by Nagappan N and John J^[24] who found chlorhexidine to be more effective against streptococcus mutans. A significant reduction of salivary streptococcus mutans count was seen 15 days after using chlorhexidine mouthwash as compared to that at a baseline level in the present study.

Oral lactobacilli are not usually affected by chlorhexidine because chlorhexidine cannot reach their ecological niches. This phenomenon has been well documented in previous studies done by Lima *et al*^[25] and Wikén Albertsson *et al*^[26].

Alum as an astringent has been recommended by the FDA’s over-the counter advisory panel as category-I active ingredient in mouthwashes^[15]. Using mouthwash with aluminium solutions has proven to impact plaque accumulation and modify the severity of pre-existing plaque in people. In contrast to the present study, Olmez *et al*^[27], and Mourughan & Suryakanth^[28] observed a significant reduction in the counts of oral Streptococci. Other studies have also

indicated alum to be an effective antibacterial agent, especially against the oral bacteria but the present study showed a good result in the reduction of plaque and calculus.

Hiora (Himalaya) mouthwash is a herbal mouthwash which is a potent oral care solution designed to combat common dental issues. Enriched with natural ingredients, this mouthwash offers a refreshing solution for plaque, gingivitis and halitosis ensuring comprehensive oral hygiene. A similar study conducted by Sharma *et al*^[29] reported that Chlorhexidine was better as compared to Hiora in reducing *S. mutans* count which supports the present study.

Terminalia chebula belongs to the family of Combretaceae which possesses anticarcinogenic properties in addition to antidiabetic, anti-inflammatory, and antioxidant effects. The cold aqueous extract was used in the present study which is in agreement with a study done by Palit *et al*^[4] to evaluate the anticarcinogenic efficacy of hot and cold aqueous extracts of *terminalia chebula* against *Streptococcus mutans* as an oral rinse and concluded that both types of aqueous extract of *terminalia chebula* may be used as potential anticarcinogenic mouthwash with acceptable taste in children. Preparation of hot extract was incorporated by Nayak *et al*^[16], while the methodology of Carounanidy *et al*^[30] was followed to prepare cold aqueous extract.

Due to the binding of chlorhexidine to soft and hard tissues, its effect seemed to sustain for a longer period of time after discontinuation of mouthwash as compared to herbal mouthwash. *Terminalia chebula* extract mouth rinse was found to be the second most significantly effective antibacterial mouth rinse next to chlorhexidine mouthwash when compared to the other studied mouthwashes.

CONCLUSION

From the results of the present study, it can be concluded that chlorhexidine is the most effective antibacterial mouthwash among the four studied mouthwashes. The study demonstrated that alum-containing mouthwash improved plaque inhibition. However, herbal mouth rinses such as Hi-ora and *Terminalia Chebula* have shown promising results, and thus, they can be considered as a newer alternative. *Terminalia Chebula* may be used as an effective oral rinse in children with high caries risk to reduce the oral load of *Streptococcus mutans* with aromatic and cooling properties, no side effects and better shelf life.

As it has been wisely said “Prevention is better than cure,” we should emphasize more towards prevention of dental caries by using nutraceutical products which is a safe alternative and possess efficient quality.

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