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Comparative evaluation of the anti-cariogenic efficacy of Coleus forskohlii root extract, cranberry extract and magnetized water as mouthrinses in 6-12 years old children: An in vivo study

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Highlights

- Coleus forskohlii root extract shows the maximum reduction in the S. mutans count and an increase in the salivary pH and buffering capacity, thus acting as a anti-cariogenic agent.
- Cranberry extract and magnetized water also showed a relative decrease in the S. mutans count and a favourable pH and buffering capacity of the saliva.
- Anti-cariogenic compounds like Coleus forskohlii root extract, cranberry extract, and magnetized water have demonstrated their effectiveness in improving oral health.

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Abstract

Aim: To evaluate and compare the anti-cariogenic efficacy of Coleus forskohlii root extract, cranberry extract and magnetized water as mouthrinses by analysing pH and buffering capacity of saliva and Streptococcus mutans count in 6-12 years old children. **Methods:** The current study was an in vivo experimental study where 120 children were randomly assigned to four groups of 30 each. Group 1: Coleus forskohlii root extract, Group 2: Cranberry extract, Group 3: Magnetized water, Group 4: Chlorhexidine mouthrinse. Unstimulated saliva was collected and tested for salivary pH, buffering capacity and S. mutans count at 0, 30, 60, and 90 minutes after rinsing and the results were evaluated. Comparison between groups was carried out using one-way ANOVA, independent t test and paired t test. **Results:** While magnetized water showed the maximum alteration in the salivary pH, Chlorhexidine mouthrinse showed the maximum reduction in the microbial count followed by Coleus forskohlii root extract. All the groups maintained a favourable salivary buffering capacity. **Conclusions:** Coleus forskohlii root extract, cranberry extract and magnetized water have proven to be effective anti-cariogenic agents and can be used as alternatives to chlorhexidine in improving the oral health with added systemic benefits and minimal side effects.

Keywords: Chlorhexidine; Coleus Forskohlii; Mouthwashes, Vaccinium Macrocarpon

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INTRODUCTION

The exponential advancements in the field of dental cariology have re-emphasized the importance of prevention. The use of mouth rinse may be a helpful addition to the oral hygiene routine, since they offer the benefit of reaching areas not easily accessed by a toothbrush.[1] Chemical plaque control has been recommended as an alternative and adjunctive to mechanical plaque control in the children, especially with special health care needs.[2] The majority of the antimicrobial agents currently available for the treatment of dental caries are broad-spectrum antibiotics.[3] Thus, these kinds of mouth rinses cannot be recommended for children younger than 6 years of age, because swallowing reflexes may not be well developed in younger children. They may swallow large amounts of the mouthrinse, which can trigger adverse events like nausea, vomiting, and intoxication due to the alcohol content in some rinses.[4,5]

Chlorhexidine, one such religiously prescribed mouthwash, is still considered as the 'gold standard' for its high substantiveness and its potent action against microbes; its side effects such as hard and soft tissue pigmentation, alteration in taste, burning sensations, staining and the formation of supragingival calculus restricts its continued use.[6,7] An ideal chemotherapeutic plaque control agent should be specific, substantiate, stable and free of adverse effects. But none of them possess all of these characteristics. Owing to the absence of new generation antibiotics and their high expense, there has been an increase in the search for alternative phytomedicines.[6] Thus, a clinical trial has been undertaken to comparatively evaluate the anti-cariogenic efficacy of three mouth rinses derived from *Coleus forskohlii* root extract, Cranberry extract and magnetized water.

Coleus forskohlii belonging to the mint family *Lamiaceae*[8] possesses potent antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumonia* etc due to the phytochemicals present in it.[9,10] An active ingredient of the American cranberry (*Vaccinium macrocarpon*), nondialyzable material (NDM), has proven to reduce the ability of the bacteria to form biofilm when it adheres to it. Antibacterial properties of the cranberry are attributed to proanthocyanidins and flavanols, which act against *Streptococcus mutans*. Research indicates that cranberries contain polyphenols that may help prevent and/or treat dental caries and help improve periodontal health.[11] Magnetic fields alter the electrical characteristics of hydrogen ions and minerals in water when they pass through them.[12] Under anaerobic conditions, the ferrite magnet caused a strength dependent decrease in growth rate and number of bacteria when grown for *S. mutans* and *S. aureus*.[13]

Though, *Coleus forskohlii* root extract, Cranberry extract and magnetized water have proven to be efficient agents in medical field, scant data are available to prove their efficiency in improving oral health, especially in children. Thus, the current *in vivo*, double-blinded, randomized parallel group clinical study has been formulated to comparatively evaluate the anti-cariogenic efficacy of *Coleus forskohlii* root extract, Cranberry extract and magnetized water as mouthrinses in 6-12 years old children.

METHODS

Ethical approval

The current randomized comparative study was conducted in an orphanage. An informed written consent was obtained from the officials of the organization prior to the study. The study was reviewed and approved by the ethical board of the university. (Ref. no. -PDCH/20/EC-217) This study has been registered under the Clinical Trials Registry by the registration number CTRI/2021/07/034954. The study was adhered

to the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Sample size estimation

Sample size of 120 was estimated using G Power software. An α- error was fixed at 0.05 (5%) and power at 0.8 (80%) was calculated after obtaining data from similar study conducted by Carounanidy et al.[14] The sample size of 30 per group was estimated.

Selection of subjects

A total of 300 children aged 6-12 years of age residing in the orphanage were screened for the study. 120 healthy children with a dmft/DMFT index score equal to or less than 4 were included based on the inclusion and exclusion criteria. Systemically healthy children in their mixed dentition with no history of antibiotics or use of any mouthwash in the previous one month were included in the study. The patient should not have any fixed or removable orthodontic appliances or removable prosthesis. Children with the abscess, draining sinus, cellulitis or other conditions requiring emergency medical treatment and those with severe malalignment of the teeth were excluded from the study.

These children were randomly divided into 4 groups. Initially 45 children were allotted to each group by lottery method. Considering the samples lost to follow-up and those that were not suitable for analysis, a sample size of n=30 was maintained in all the four groups. (Figure 1):

Group 1: *Coleus forskohlii* extract

Group 2: Cranberry extract

Group 3: Magnetized water

Group 4: Chlorhexidine (control)

Preparation of the mouthrinses

Coleus forskohlii

One kg roots of *Coleus forskohlii* were taken, cleaned and shade dried. After drying the roots were grounded using mortar and pestle. In a 1L beaker, 50g of the powdered plant material was soaked in 70 percent of 100 ml ethanol. The beaker was wrapped in aluminium foil before placing it in a solidicator for 30 minutes to prevent contamination. Whatman filter No.1 was utilised for obtaining a particle-free extract. The filtrate was then concentrated at 45°C under decreased pressure in a rotary evaporator, yielding crude extract that was diluted with distilled water. The extract was prepared at the concentration of 0.5% using xylitol as a sweetening agent

Cranberry extract

Cranberry extract was procured from dried cranberries. Since Sethi et al.[15] showed the greatest zone of inhibition at 0.6% of cranberry against *S. mutans*, in the current study same concentration has been chosen to prepare the mouthwash. 100 ml of cranberry mouthwash was prepared by dissolving 600 mg of cranberry extract in 100 ml of distilled water using xylitol as a sweetening agent.

Magnetized water

Magnetized water was prepared by placing magnets over reverse osmosis (RO) water for 72 hours. The procedure of magnetization of water and the time of magnetization of water was in accordance with a study conducted by Gupta and Bhat,[6] and Goyal et al,[13] where they found that 72 hours of magnetization showed reduction in *S. mutans* approximately similar to 0.2% chlorhexidine when compared to 24 hours of magnetization.

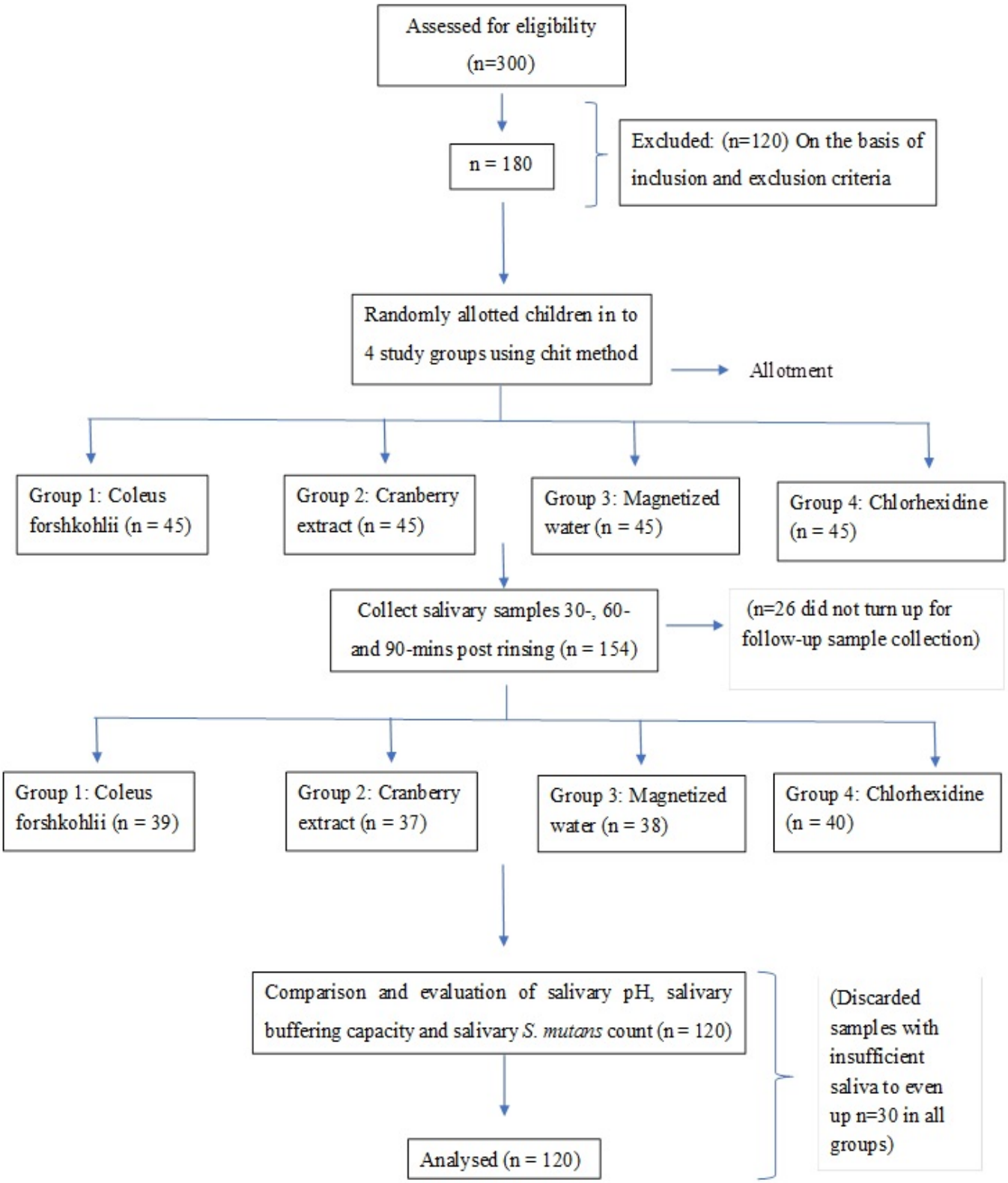

Figure 1. Flow chart of clinical trial procedure and randomization according to CONSORT guidelines

Collection and analysis of saliva samples

The unstimulated saliva obtained on chewing a piece of paraffin wax for 30 seconds was collected for testing. The children were asked to rinse for one minute with 10 ml assigned mouth rinse, 10 minutes after the baseline sample collection.

The samples of unstimulated saliva were collected again at 30-, 60- and 90-minutes post rinsing. The pre-rinse and post-rinse samples (after 30-, 60- and 90- minutes) were collected and tested for salivary pH, salivary buffering capacity and salivary *S. mutans* count.

Salivary pH analysis

Salivary samples obtained from the 120 patients were analysed using Indikrom pH paper strips. The pH test paper was immersed in the sample for at least 10 seconds and the color changes were compared with the manufacturer's chart. The pH values were noted.

Salivary buffering capacity analysis

The GC saliva check kit was used to evaluate the salivary buffering capacity. One drop of saliva was placed on each test pad using the pipette provided. The colour of the strip was compared with the manufacturer's chart after 2 minutes, and the values were noted.

Salivary microbial analysis

The dilution and spread plate technique were used for assessing the microbial content. The samples were diluted using saline and then streaked on to petri plates containing the Mitis salivarius-bacitracin (MSB) agar for *S. mutans*. The colonies were counted after incubating the plates for 72 hours at 35°C. Patients were not allowed to rinse with water or to consume anything orally throughout the procedure. The pH and buffer test were repeated at 30-, 60- and 90-minutes intervals. The microbial analysis was repeated in 90 minutes, and the results were recorded.

Statistical analysis

Data were entered into an Excel file (Microsoft 2010) and checked for accuracy. The data analyses were carried out using SPSS 21(Statistical Package for the Social Sciences, Inc., Chicago, Illinois, USA). Comparison between groups will be carried out using one-way ANOVA, independent *t* test and paired *t* test. Level of significance was fixed at $p \leq 0.05$ and any value less than or equal to 0.05 was considered to be statistically significant**.**

RESULTS

The values of salivary pH and salivary buffering capacity as well as salivary microbial count of all the groups at the baseline and after 30, 60 and 90-minutes after rinsing are summarized as Figures 2,3 and 4. The magnetized water showed the capacity to increase the pH after 90 mins of rinsing. The mean buffering capacity were found to be 7.17, 6.68, 6.71 and 7.16 respectively in groups 1,2,3 and 4.

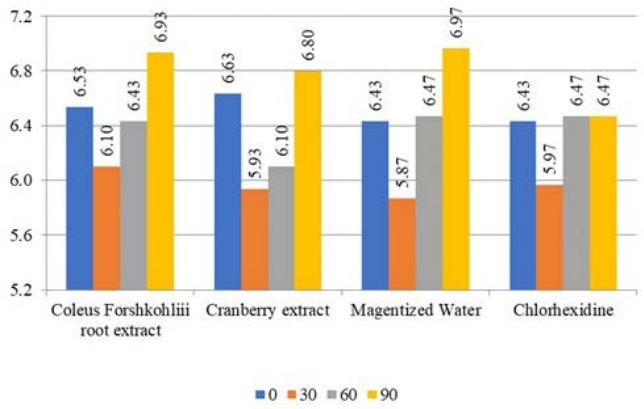

Figure 2. Graphical representation of mean salivary pH levels of the test groups at different time intervals

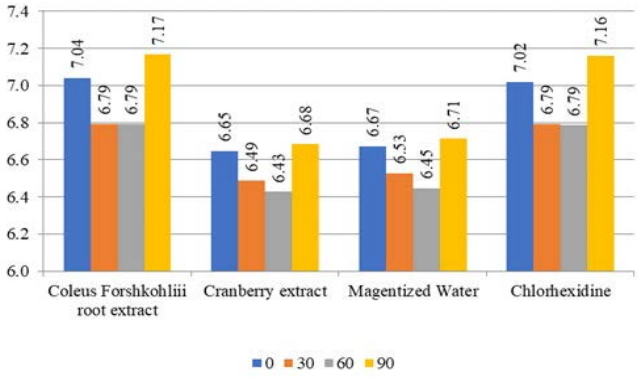

Figure 3. Graphical representation of mean salivary buffering capacity of the test groups at different time intervals

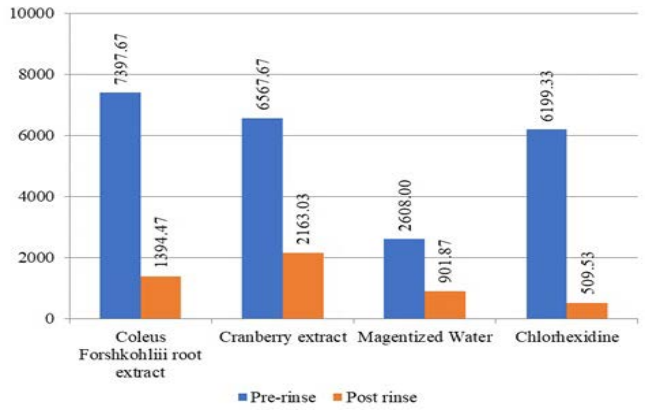

Figure 4. Graphical representation of mean salivary *S. mutans* count of the test groups at different time intervals

The current study also revealed that the children rinsed with the chlorhexidine showed the least microbial count after 90 mins. The maximum difference in mean microbial count pre-rinse and post-rinse was shown by *Coleus forskohlii* root extract. In groups 1,2,3,4 mean microbial count were 1394.47, 2163.03, 901.87 and 509.53 respectively.

DISCUSSION

One of the fundamental strategies in the current paediatric dental practice is prevention of dental caries. Hence, over the past decade advanced treatment and management protocols for dental caries have been introduced. Since mouth rinses are widely used as an adjunct to maintain oral hygiene, it's efficiency and safety is crucial for their regular use in children.[16-19] Medicinal Coleus (*Coleus forskohlii*) root extract has high therapeutic value and is safe for use in children as no harmful heavy metals were found in the roots. In the present study, while all the mouthrinses maintained the pH of the saliva, *Coleus forskohlii* root extract raised the pH to 6.73 thus maintaining a non-acidic oral environment. *Coleus forskohlii* root extract showed a reduction of 81% in salivary *S. mutans* count which clearly displayed its potency among the herbal mouthrinses. This could be attributed to the presence of Forskolin which is the major compound of the roots having high therapeutic value.[20]

Table 1. Evaluation of mean difference in salivary pH between and within the groups

		Sum of Squares	Df	Mean Square	F	Sig.
pH 0	Between Groups	0.825	3	0.275	1.094	0.35 (NS)
	Within Groups	29.167	116	0.251		
pH 30	Between Groups	0.867	3	0.289	0.657	0.58 (NS)
	Within Groups	51.000	116	0.440		
pH 60	Between Groups	2.867	3	0.956	2.262	0.08 (NS)
	Within Groups	49.000	116	0.422		
pH 90	Between Groups	4.692	3	1.564	12.014	0.000*
	Within Groups	15.100	116	0.130		

One way ANOVA test; *indicates significant at $p \leq 0.05$ NS: Non-significant

Table 2. Evaluation of mean difference in salivary buffering capacity between and within the groups

		Sum of Squares	df	Mean Square	F	Sig.
Buffering Capacity 0	Between Groups	4.124	3	1.375	8.342	0.000*
	Within Groups	19.113	116	0.165		
Buffering Capacity 30	Between Groups	2.489	3	0.830	4.063	0.009*
	Within Groups	23.691	116	0.204		
Buffering Capacity 60	Between Groups	3.752	3	1.251	5.416	0.002*
	Within Groups	26.787	116	0.231		
Buffering Capacity 90	Between Groups	6.501	3	2.167	11.586	0.000*
	Within Groups	21.695	116	0.187		

Table 3. Evaluation of mean difference in salivary *S. mutans* count between and within the groups

		Sum of Squares	df	Mean Square	F	Sig.
Microbial Count Pre-rinse	Between Groups	403335856.67	3	134445285.56	4.735	0.004*
	Within Groups	3293913340.00	116	28395804.65		
Microbial Count Post-rinse	Between Groups	45712391.56	3	15237463.85	5.115	0.002*
	Within Groups	345554771.37	116	2978920.44		

One way ANOVA test; *indicates significant at $p \leq 0.05$

Cranberry contains certain phytochemicals that exhibits promising biological effects against critical virulence properties of caries causing bacteria.[21,22] In the current study, cranberry extract showed a 2.5% increase in pH at 90 min where as the buffering capacity did not show a significant difference throughout the time intervals. The active components of cranberry are proanthocyanidins and flavonols which act against *S. mutans* and is believed to display the reversal of the majority of bacterial coaggregation; cariogenic micro-organisms when precoated with NDM shows diminished ability to form biofilm.[11] Thus cranberry extract showed 67% decrease in *S. mutans* count post-rinse, similar to findings of the study conducted by Khairnar et al.,[3] in which there was 68% reduction in *S. mutans* count after use of cranberry mouthwash.

Magnetized water showed a significant increase in pH i.e., 7.7% over a period of 90 minutes but the buffering capacity did not show any significant increase. The maximum change in the pH could be attributed to the fact that magnetized water is more alkaline due to the presence of more hydroxyl (OH–) ions that form alkaline molecules which reduce the acidity and raises the pH of our body, which allows the body to get rid of the toxins. On the contrary, it showed the least reduction in *S. mutans* count i.e., 65%; similar to the findings by Gupta and Bhat.[6]

It is believed that magnetized water does not possess inherent anti-microbial properties. When some oxygen ions within the water combine together and become oxygen molecules, it will dissolve immediately. Water having an alkaline property with oxygen inside gives energy to the cells, further preventing the development and inhibiting the growth of anaerobic bacteria.[13]

In the present study, chlorhexidine did not show a significant alteration in the pH and

buffering capacity rather it seemed to maintain a stable pH. Thus, overall comparison showed no significant difference in the buffering capacity between the study groups. Although no significant changes were seen in these parameters, the *S. mutans* count showed a drastic decrease of 91% which was maximum amongst the study groups. *Coleus forskohlii* root extract showed the closest reduction in the *S. mutans* count when compared to the control group i.e., Chlorhexidine. From the present study, it is evident that chlorhexidine is indeed a "gold standard" for caries prevention but due to certain disadvantages a potent alternative to chlorhexidine has to be looked for. The limitations of this study were that pH papers were used instead of a digital pH meter which could have given comparatively accurate readings. The pH meter could not be used due to economical restrictions. In the present study, the *Coleus forshkohlii* root extract was prepared in alcohol base, further studies can be undertaken by preparing it in aqueous base and evaluating the anti-cariogenic efficacy thus making it safer for use in children. The study was conducted on a limited sample size on a single day at different time intervals. Future scope lies in long term research trials with larger sample size and wider age group to check the efficacy and effectiveness of herbal products over standard drug regime. Within the limitations of this clinical trial, natural mouthwashes have demonstrated nearly similar effects on minimizing *S. mutans* count as compared to the synthetic mouthwash chlorhexidine. The current study advocates that herbal mouth rinses can prove to be an efficient alternative to Chlorhexidine in improving the oral hygiene without any side effects.

CONCLUSIONS

Within the limitations of the current *in vivo* clinical trial, it can be concluded that *Coleus forskohlii* root extract shows the maximum reduction in the *S. mutans* count and an increase in the salivary pH and buffering capacity, thus acting as a potent anti-cariogenic agent. Cranberry extract and magnetized water also showed a relative decrease in the *S. mutans* count and a favourable pH and buffering capacity of the saliva. Anti-cariogenic compounds like *Coleus forskohlii* root extract, cranberry extract, and magnetized water have demonstrated their effectiveness in improving oral health and can be used as alternatives to chlorhexidine while providing additional systemic benefits.

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Declarations

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