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International Journal of Oral Health Dentistry

Journal homepage: www.ijohd.org

Original Research Article

Comparative evaluation of antimicrobial efficacy of triphala, assa-foetida and 0.2% chlorhexidine mouth rinses on salivary streptococcus mutans

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ARTICLE INFO

Article history:

Received 21-12-2023

Accepted 20-01-2024

Available online 09-04-2024

Keywords:

Triphala

Asafoetida mouth rinse

Salivary Smutans

Antimicrobial efficacy

ABSTRACT

Background: Oral diseases, such as dental caries and periodontal diseases are major worldwide oral health problems. Poor oral health has a profound effect on general health and quality of life. Dental caries is still a major health problem in most industrialized countries, school-aged children and the vast majority of adults. Streptococcus mutans is considered as one of the most important cariogenic species of the human oral microbial flora. Studies have proved the association between S. mutans and dental caries. Traditional Indian system of medicine "Ayurveda" includes several important medicinal herbs, which are used since ancient days. Triphala, which has a wide range of systemic benefits, is one among them. Studies have shown that Triphala has good antimicrobial effects against S. mutans. Ferula assa-foetida (F.assa-foetida), is a large herbaceous perennial plant from the Apiaceae family. The properties of Assa-foetida according to Traditional Persian Medicine resources suggest it is very effective for relieving toothache. Different parts of F. assa-foetida have a wide variety of medicinal applications including antifungal, anti-diabetic, anti-inflammatory, anti-mutagenic and anti-microbial effects.

Aim: To compare and evaluate the antimicrobial efficacy of Triphala, Assa-foetida and 0.2% Chlorhexidine mouth rinses on the salivary Streptococcus mutans level.

Material and Methods: Saliva samples were collected from children of age group of 5-11 years with a DMFT/def score ≥ 4 and with minimum of four active caries. Triphala and Asafoetida mouth rinses were prepared indigenously. Samples were plated on the culture media and then smeared on MHA media for assessing the antimicrobial efficacy against Chlorhexidine. The zone of inhibition was measured which showed the resistance against S.mutans.

Results: Results were statistically analyzed using one way ANOVA. Significant resistance was noted with asafoetida mouth rinse by measuring the zone of inhibition.

Conclusion: Considering the results, Triphala and asafoetida mouth rinses serve as effective herbal mouth rinses for reducing the salivary Streptococcus mutans load and can be considered as an alternative to chemical mouthwash, like Chlorhexidine.

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1. Introduction

Oral Conditions, such as dental caries and periodontal conditions are major worldwide oral health challenges. Poor oral health has a profound effect on general

health and quality of life.¹ Dental caries, an contagious microbiological disease of teeth, is still a major health problem in most industrialized countries, as it affects 60 to 90 of school-aged children and a vast majority of adults.^{1,2} Streptococcus mutans is considered as one of the most dominant cariogenic species of the human oral microbial flora and is the causative agent for

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initiation of dental caries.³They're gram-positive cocci, immotile, facultative, anaerobic microorganisms, which can metabolize carbohydrates and are considered to be the primary etiological agent of dental caries. It's a crucial contributor to the formation of cariogenic plaque because this bacterium efficiently utilizes dietary sucrose to create large amounts of extracellular polysaccharides adheres steadily to glucan-coated surfaces, and is also largely acidogenic and acid-tolerant.¹For the prevention of dental caries, mouth rinsing in children and adolescents was established as a mass prophylactic technique in the 1960s and had shown an average efficacy of caries reduction between 20 and 50.¹Chlorhexidine is the gold standard for chemotherapeutic agents against Streptococci mutans and dental caries and has been studied extensively for over 20 years.¹Reduction of the number of cariogenic bacteria should be the primary aim of a caries prevention program. In 1986, Loesche described caries and periodontal disease as "maybe the most expensive infections that most individuals have to contend with during a lifetime." However, the adverse side-effects of chlorhexidine have urged to look for herbal alternatives.³The effect of mechanical oral hygiene styles on the salivary microorganisms, especially Streptococcus mutans, is of great interest to dentists concentrating on preventative care. Tooth brushing with fluoridated toothpaste is believed to be the bed-rock of caries prevention. However, tooth brushing alone is effective in reducing bacterial counts in the mouth.⁴Traditional Indian system of drug " Ayurveda " includes several important medicinal herbs, which are used since ancient days. Triphala, which has a wide range of systemic advantages, is one among them. Triphala is an Indian Ayurvedic herbal formulation that consists of dried and powdered fruits of three medicinal plants called Terminalia bellerica, Terminalia chebula and Emblica officinalis.^{3,5}Studies have shown that Triphala has good antimicrobial properties against S. mutans. Triphala exerts significant cardioprotective, cardiotonic and hepatoprotective effects. Mouthwash containing ethanol extract of Terminalia chebula has considerable antibacterial activity against Streptococcus mutans. It's a safe, effective and economical anticaries agent. Mouthwash containing aqueous extract of 10% Terminalia chebula, increases the pH and buffering capacity of saliva. Also the counts of S. mutans and Lactobacilli are diminished.⁶Ferula assa-foetida(F.assa- foetida), is a large herbaceous perennial plant from the Apiaceae family. The properties of Assa-foetida according to Traditional Persian Medicine coffers suggest it's veritably effective for relieving toothache. Different parts of F.assa foetida have several of medicinal uses including antifungal, anti-diabetic, anti inflammatory, anti-mutagenic and anti-microbial effects. Kavousi et al. in 2013 have established its antioxidant and anti-microbial effects against different aerobic and nonaerobic bacteria.

Also, in a review study by Iranshahi et al. in 2011, the authors confirmed the anti-inflammatory and anti-microbial effects of F. Assa- foetida. Assa- foetida has a sound effect on improving gingival health.^{5,7}The focus of exploration is shifting towards further natural products or herbally derived antibiotic products in general and specifically in the prevention of dental caries. The global need for options for prevention and treatment choices that are safe, effective, and provident arise due to increased incidence of conditions (particularly in developing countries), elevated resistance by pathogenic bacteria to presently used antibiotics and chemotherapeutics. Several commercially available chemical antiplaque agents can be delivered in the form of mouthwash, dentifrices, chewing gums, and gel. However, they've some undesirable side effects such as vomiting, diarrhoea, and tooth staining.⁸The studies showed high efficacy of Triphala mouth rinse versus chlorhexidine against S. mutans; many studies have done to demonstrate the effects of Assa- foetida on the oral microorganisms. As there's need for a herbal alternative and veritably many studies were done on the effectiveness of Assa- foetida against salivary Streptococcus mutans, this study aims to evaluate the efficacy of Triphala and Assa- foetida mouth rinses and to compare it with 0.2% chlorhexidine on the salivary S.mutans.

2. Aim

To evaluate the antimicrobial efficacy of Triphala and Assa-foetida mouth rinses and to compare it with 0.2% Chlorhexidine on the salivary S.mutans.

3. Material and Methods

This was an experimental study conducted to evaluate the effectiveness of antimicrobial efficacy of Triphala and Assa-foetida mouth rinses and to compare it with 0.2% Chlorhexidine on the salivary S.mutans. The study was approved by the Institutional Ethical committee and informed written consent was obtained from parents and participants after explaining the study.

3.1. Inclusion criteria

Children of 5-11 years, a minimum of 4 active caries lesions

3.2. Exclusion criteria

Patients who had consumed antibiotics in the past month before the onset of the study and those with any systemic diseases.

3.3. Methodology

A total of 31 patients, aged 5-11 years of both gender from the patients reporting to the Department of Pediatric and Preventive Dentistry were included in the study. Assent

forms from the children and signed consent from the parents were taken after explaining the procedure of the study in detail.

A brief case history was recorded for ruling out any systemic diseases and any history of antibiotic consumption.

Children having a minimum of 4 active caries lesions were recruited for the study.¹ Saliva samples from these children were collected in dry sterile containers and stored in an icebox till the time of transferring the samples for further analysis.

3.4. Collection of saliva sample

Approximately 4 mL of unstimulated salivary samples were collected. Following standard procedures, subjects were asked to wash their mouth and sit passively as the saliva accumulated on the floor of the mouth and expectorate in a relaxed position with their heads bent forward and spit into a dry sterile container.⁹ The collected saliva samples were stored in an icebox before transferring for the microbial analysis (Figures 1 and 2).

3.4.1. Preparation of triphala mouth rinse

A quantity of 10 gm of Triphala churna was dissolved in double de-ionized water and was boiled and filtered. To the filtrate, 2mL of glycerine as a sweetening agent and 1 mL of Pudina Hara were added as a flavoring agent. Then the solution was cooled and 50mL was measured and dispensed in amber colored bottles.¹ (Figure 3)

3.4.2. Preparation of assa-foetida mouth rinse

A quantity of 10 g of Asa-foetida powder was dissolved in 100cc of water and then mixed in a 250ml Erlenmeyer flask. The solution was then placed on a stirrer for 24h. Then, it was filtered by a vacuum pump and an Atman filter. The solution was then mixed with water by 0.5%w/w and 1 mL of Pudina Hara were added as a flavoring agent and packaged in amber colored bottles.¹⁰ (Figure 4)

3.5. Chlorhexidine (0.2%)

A commercially available mouth rinse (HEXIDINE®, manufactured by ICPA Health Products Limited) was used for the study as control. A 50 mL of mouth rinse was dispensed in an amber-colored bottle.¹ (Figure 5)

3.6. Microbiological assessment

The collected unstimulated saliva sample was mixed vigorously for 30 seconds to ensure a representative mixture throughout the sample before the preparation of dilutions and plating. After this, 1ml of saliva was added to 9ml of sterile saline and mixed. Then this mixture was serially diluted to prepare suspensions of 10^{-1} , 10^{-2} and 10^{-4} concentrations. From 10^{-1} , 10^{-2} and 10^{-4} concentrations, 100 μ L was plated onto Mitis salivarius bacitracin (MSB)

agar (Figure 6) for culturing of salivary Streptococcus mutans. The prepared plates were incubated at 37°C for 48 hours. The counts of the colonies (Figure 8) were counted using the colony counter. One colony (pure) was taken and a lawn culture was prepared on Mueller-Hinton agar (Figure 7) for testing the antimicrobial susceptibility of the mouth rinses. Three wells (10 mm) were made on agar plates and 100 μ L of each Triphala, Assa-foetida and chlorhexidine (control) mouth rinses were loaded into the wells respectively. The plates were incubated aerobically at 37 °C for 48 hours. The zone of inhibition (Figure 9) was measured in millimetres (mm) using a HiAntibiotic Zone Scale-C (Figure 10) and further analysis was done based on Clinical and Laboratory Standards Institute (CLSI) guidelines to derive the conclusion.

3.7. Statistical analysis

At a 1% level of significance and the standard deviation is 0.58⁷ with 2% of precision around the mean 0.27, the total sample size is 31. Descriptive statistics was performed for the analysis of the present study. One way ANOVA was used to test among all the mouth rinse groups. For group comparison, post hoc Tukey's HSD analysis was performed.

4. Results

The present study included 31 children (both genders) of age group 5-11 years. Saliva was collected from these children in a sterile container and sent for microbiological assessment. The number of colonies of S.mutans growth on Mitis Salivarius-Bacitracin agar, which is the selective media for S.mutans, was counted using colony counter (Table 1). The efficacy of the Triphala, Asafoetida and Chlorhexidine mouth rinses were determined with the zone of inhibition seen on the Muller-Hinton Agar plate measured using HiAntibiotic Zone Scale-C (Table 2).

After the microbiological assessment, the collected data were evaluated between the groups using one-way ANOVA test. A result with P value < 0.05 was considered as statistically significant. In the present study, post hoc Tukey's HSD analysis was performed for the multiple group comparison.



Figure 1: Saliva collection

Table 1: No. of colonies of Streptococcus mutans growth on MSB agar

Samples	Dilutions of saliva sample		
	-1	-2	-4
RS01	2 colonies	No growth	No growth
RS02	4 colonies	No growth	No growth
RS03	3 colonies	1 colony	1 colony
RS04	5 colonies	2 colonies	2 colonies
RS05	6 colonies	No growth	No growth
RS06	10 colonies	2 colonies	No growth
RS07	8 colonies	1 colony	1 colony
RS08	12 colonies	5 colonies	No growth
RS09	10 colonies	4 colonies	No growth
RS10	7 colonies	3 colonies	1 colony
RS11	3 colonies	2 colonies	1 colony
RS12	4 colonies	2 colonies	2 colonies
RS13	3 colonies	1 colony	1 colony
RS14	4 colonies	2 colonies	No growth
RS15	5 colonies	3 colonies	1 colony
RS16	4 colonies	2 colonies	2 colonies
RS17	8 colonies	4 colonies	2 colonies
RS18	5 colonies	3 colonies	1 colony
RS19	13 colonies	10 colonies	5 colonies
RS20	7 colonies	4 colonies	2 colonies
RS21	6 colonies	4 colonies	1 colony
RS22	6 colonies	3 colonies	2 colonies
RS23	7 colonies	5 colonies	No growth
RS24	8 colonies	5 colonies	2 colonies
RS25	No growth	No growth	No growth
RS26	No growth	No growth	No growth
RS27	7 colonies	6 colonies	3 colonies
RS28	5 colonies	3 colonies	2 colonies
RS29	10 colonies	6 colonies	3 colonies
RS30	No growth	No growth	No growth
RS31	No growth	No growth	No growth

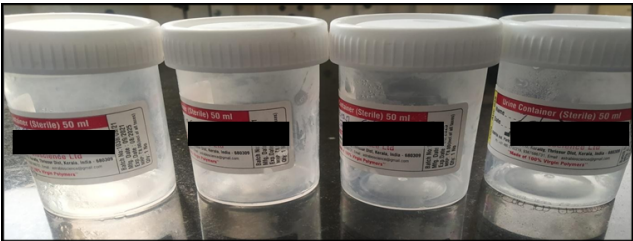


Figure 2: Collected saliva

5. Discussion

Modern conceptions believe caries as an relation between genetic and environmental factors in a largely complex and interactive manner.¹WHO supports the indigenous systems of health care which is found to be effective and helpful. Traditional herbal drugs comprises of plant derived substances with least or no industrial processing which is used to treat diseases.²Dental caries is a common oral bacterial pathology caused by a biofilm consisting



Figure 3: Prepared triphala mouthrinse

of microorganisms present on the faces of the tooth. Streptococcus mutans is the most common microorganism associated with dental caries. It's well- known that bacteria with efficient adaptive mechanisms accommodate to new environmental and biological circumstances.³In addition

Table 2: Zone of Inhibition (in mm) on MHA agar

Streptococcus mutans	T	A	C
RS 01	21mm	no zone	26mm
RS 02	13 mm	12mm	18 mm
RS 03	10mm	no zone	17mm
RS 04	12mm	10mm	24mm
RS 05	14mm	14mm	20mm
RS 06	12mm	11mm	14mm
RS 07	13mm	11mm	20mm
RS 08	26mm	27mm	22mm
RS 09	15mm	11mm	19mm
RS 10	14mm	10mm	25mm
RS 11	16mm	12mm	27mm
RS 12	16mm	15mm	23mm
RS 13	20mm	14mm	23mm
RS 14	24mm	20mm	25mm
RS 15	20mm	12mm	22mm
RS 16	12mm	10mm	21mm
RS 17	13mm	10mm	20mm
RS 18	15mm	10mm	22mm
RS 19	27mm	13mm	26mm
RS 20	20mm	14mm	24mm
RS 21	24mm	13mm	27mm
RS 22	22mm	14mm	27mm
RS 23	20mm	13mm	26mm
RS 24	No growth	No growth	No growth
RS 25	No growth	No growth	No growth
RS 26	23mm	12mm	28mm
RS 27	25mm	15mm	27mm
RS 28	24mm	13mm	25mm
RS 29	23mm	14mm	27mm
RS 30	No growth	No growth	No growth
RS 31	No growth	No growth	No growth

Table 3: Group comparison of Mean values and standard deviation

	Mean	SD	F value	p value
T	15.94	7.878		
A	10.65	6.221	12.134	0.0001
C	20.16	8.572		

p<0.05 is considered as significant.

Table 4: Multiple comparison using Tukey's HSD

	Mean Difference	p value	95% Confidence Interval	
			Lower Bound	Upper Bound
T with A	5.290	0.020	0.68	9.90
T with C	-4.226	0.080	-8.84	0.39
A with C	-9.516	0.0001	-14.13	-4.90

p<0.05 is considered as significant

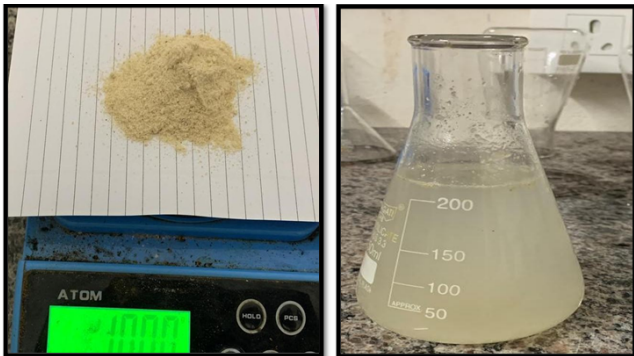


Figure 4: Prepared assa-foetida mouthrinse



Figure 5: Chlorhexidine mouthrinse

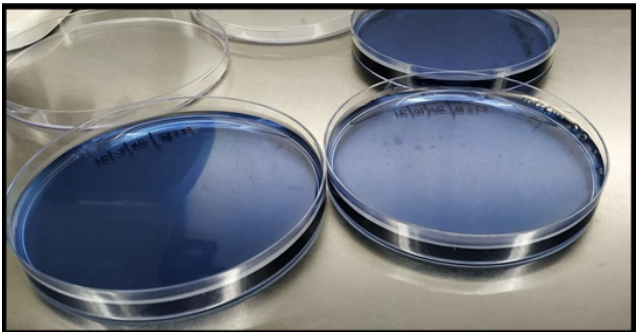


Figure 6: Prepared Mitis Salivarius Bacitracin Agar (MSB)

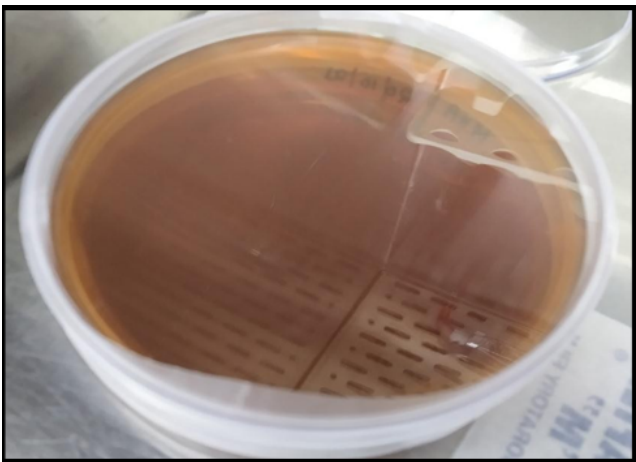


Figure 7: Prepared Muller Hinton Agar (MHA)

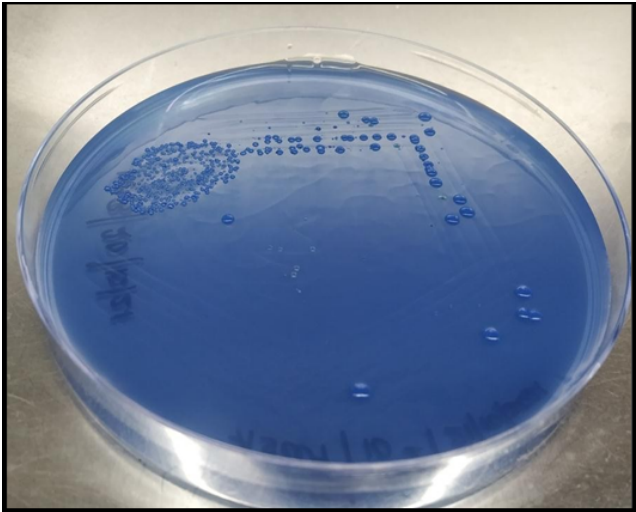


Figure 8: Streptococcus mutans growth MSB

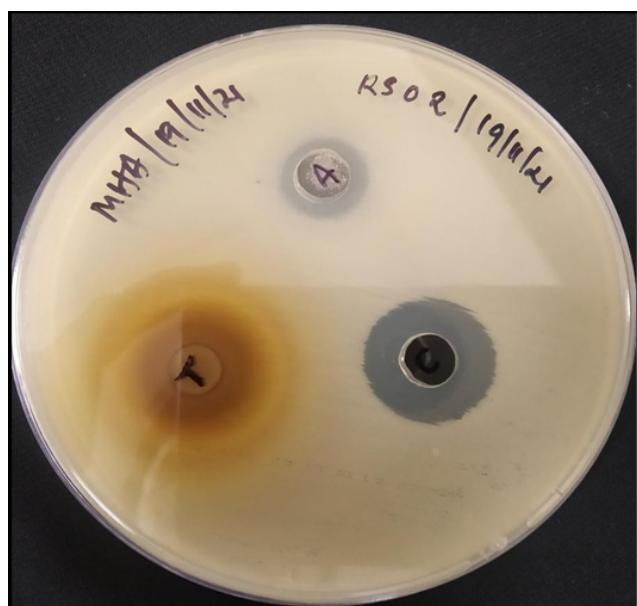


Figure 9: Zone of Inhibition on MHA media

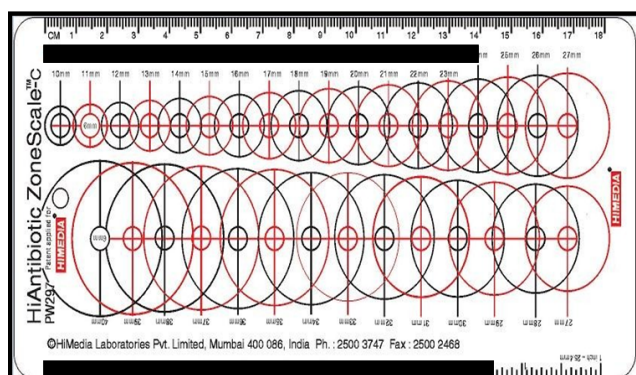


Figure 10: Hi Antibiotic Zone Scale-C

to dental caries and related pyogenic dental infection, *S. mutans* is also a veritably important endocarditis agent. The participation of the microorganism in both oral and non oral conditions has promoted interest in the knowledge of its vulnerability to antimicrobial agents.¹¹ The present study was an experimental study. Children of age 5- 11 years were included in the study as it's the mixed dentition stage, which coincides with the second window of infectivity. The second window of infectivity is observed during 6-12 years of age when multiple permanent teeth erupt and the surfaces of the tooth are exposed to caries risk.¹² In the present study, saliva was collected from children with minimum 4 active caries. Unstimulated and stimulated saliva are two different techniques of saliva collection. In this study, unstimulated saliva was collected in sterile containers. It was used because of its lower concentration of bicarbonate ions, therefore reducing the buffering action of saliva. Saliva was collected by spitting technique in

sterile containers (Figure 2). Based on the results of the present study, maximum number of colonies of *S. mutans* on MSB agar was 13 colonies (Table 1). There was a positive correlation between number of decayed teeth and the number of colonies of *S. mutans* obtained on MSB agar. L.Salonen et al and Satu Alaluusua et al. reported similar results showing a positive correlation between the concentration of mutans streptococci in saliva and dental caries. Individuals with lower concentrations showed a significantly lower mean number of decayed surfaces compared with the individuals with higher concentrations of *S. mutans* in their saliva.¹³ Even though some literature has supported a positive correlation between *S. mutans* levels and caries experience, other studies have found no clearly defined correlation between the two, suggesting that *S. mutans* alone cannot explain all caries experience.¹⁴ This explains why in the present study, there was no growth seen on MSB agar in two slaver samples. (Table 1) Chlorhexidine gluconate (CHG) is a bis- biguanide that was first described by Hassan et al. It's an antimicrobial agent with a long history as a substance for inhibiting plaque formation and has a special affinity for oral structures. Chlorhexidine has been considered as the “ gold standard ” among the chemical plaque control techniques and is largely effective in reducing the oral microbial load.¹ Considering the adverse effects of the use of chlorhexidine, its use for long term therapy has been limited or not actively recommended.⁶ Chlorhexidine showed statistically significant by the study of Emilson where it was found that Chlorhexidine treatment reduces *S. mutans* counts for a period 4- 6 months.¹⁵ The adverse effects have been attributed to the use of mouthwashes currently available in the market, such as taste revision, unpalatable taste and increase threat of caries due to fermentation and alcohol content, and discoloration of teeth. This has increase the research for alternatives that are more appropriate for young children. One approach to solve this problem is through the use of home- made remedies that are readily accessible, secure, useful, and suitable to all.¹ Therefore in this present study, we assessed the efficacy of indigenously prepared Triphala and Assa- foetida mouth rinses using commercially available powders and compared it to 0.2% Chlorhexidine mouthwash. In this present study, indigenously prepared Triphala mouth wash was evaluated for its efficacy to reduce the salivary *S. mutans* level. As reported by Susruta Samhita, Triphala can be used as a gargling agent in dental diseases. Triphala have been used to treat many systemic diseases by Ayurvedic practitioners. No evidence of harmful effects of Triphala on swallowing has reported in any literature. The ingredients used to prepare Triphala are the frequently available fruit derivatives.^{16–25} It's prepared by mixing the dried and powdered mixture of Triphala constituted by amla, vibhitakai and haritakai in a 1:1:1 ratio which is quite economical. Triphala is

a strong antioxidant and antimicrobial agent and can be helpful in oral cavity infections like dental caries, gingivitis and oral candida infections. It has an important role in preventing dental caries by strongly inhibiting the salivary *S. mutans*. The effectiveness of Triphala mouthrinse was as good as the standard Chlorhexidine mouthrinse (0.2%). In limited samples, Triphala showed more effectiveness than Chlorhexidine. As stated by Khorana et al in their study, there was significant inhibitory effect of Triphala mouthwash on *Streptococcus mutans* growth.¹⁵ In this study, indigenously prepared *Assa- foetida* mouth rinse has showed significant effect on reducing the salivary *Streptococcus mutans* load. The results showed that this can be similar, but less effective to that of Chlorhexidine mouthrinse. *Assa- foetida* mouthrinse on MHA agar showed significant zone of inhibition against *S. mutans*. However, the concentration of the mouth rinse used in the present study seems to be less.^{26–30} In comparison with Triphala and Chlorhexidine zones of inhibition, *Assa-foetida* showed lower zone of inhibition on MHA agar. These results are corresponding to the results reported by Kavooosi and Rowshan and Siddiqui et al although these two studies have assessed the antimicrobial effect of essential oil obtained from *Ferula oleo- gum- resin* on *Staphylococcus aureus*, *Bacillus Subtilis*, and *Escherichia Coli*.³¹ They also found the antioxidant and antibacterial activities of *Ferula assa- foetida* and showed that this has inhibitory effects on the growth of gram-positive bacteria. The antimicrobial activity of *Ferula assa- foetida* may be due to biological active compounds. Also, *Assa-foetida* possesses anti-inflammatory property which is a favourable characteristic for treatment of different dental and gingival disorders. Moreover, the aqueous extract of the *asafoetida* is effective in increasing the proliferation of epithelial cells and speeding up blood flow in the inflammatory processes.⁷ Holistic dentistry is an alternate approach which is an emerging field of dental medicine and more researches need to be conducted in this field. Holistic dentists are also recognized as biological or environmental dentists. They operate according to the idea that your tooth is a vitally important part of your body, and hence affect your overall health in profound ways that we may not yet completely appreciate. Practitioners of holistic dentistry may vary quite a bit in terms of the services they offer and the approaches they take to dental care. Several chemical anti-plaque agents are available commercially, and they can be delivered in the form of mouthwash, dentifrices, chewing gums, and gel.⁶ The extended use of these chemical anti-plaque agents are limited due to original side goods including extrinsic tooth and tongue brown staining, taste disturbance, enhanced supragingival calculus formation, and desquamation of the oral mucosa. On the other hand, herbal mouthwash due to its natural ingredients has no reported side effects and can serve as a good alternative. The major advantages of

herbal alternatives are easy accessibility, cost- effectiveness, increased shelf life, low toxicity and lack of microbial resistance reported so far.³ According to the present study, herbal alternatives seem to be effective and safer in children for the management of dental conditions. Further clinical investigations for standardization and preparation of mouthwashes containing the herbal antimicrobial agents for the prevention of oral microbial conditions, are needed to confirm their use in children.

6. Conclusion

Based on the results of the present study, the herbal mouth rinses, Triphala and *Assa-foetida*, have shown statistically significant reduction of salivary *Streptococcus mutans* load. The efficacy of *Assa-foetida* mouth rinse was lesser when compared to Triphala and Chlorhexidine. The comparative evaluation of the herbal mouth rinses showed that they can be considered as effective alternatives in improving oral health. Between the herbal mouth rinses, Triphala showed a higher efficacy than *Asafoetida*. *Asafoetida* also has a good effect in reducing the *S. mutans* level in the saliva. The herbal mouth rinses with less adverse effects are better alternatives which can safely be used in children. Evidences from the literature have proved the antimicrobial efficacy of Triphala, but fewer evidences on *Assa-foetida* mouth rinses are available. Although, *asafoetida* mouth rinse can be considered as a promising alternative to chemical mouthwashes, further studies are required to test the antimicrobial efficacy against biofilm and other microorganisms with different concentrations of *asafoetida* mouth rinses.

7. Source of Funding

None.

8. Conflict of Interest

None.

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Cite this article: Simon R, Ajay Rao H.T, Sargod SS, Suvarna R, Shabbir A. Comparative evaluation of antimicrobial efficacy of triphala, assa-foetida and 0.2% chlorhexidine mouth rinses on salivary streptococcus mutans. *Int J Oral Health Dent* 2024;10(1):19-27.