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Evaluation of Antibacterial Effect of Triphala Plant Extract on Early Colonizers of Dental Biofilm

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KEYWORDS

Triphala , Chlorhexidine , Dental plaque.

ABSTRACT:

Aim : the objective of this study was to access the antimicrobial effect of triphala mouthwash on early colonizers of dental plaque and compare it with commercially available chlorhexidine mouthwash

Material and method : A total of 30 patients in the age group 8-12 years were the subjects of this study. The solutions were divided into Group I Chlorhexidine (0.2%- positive control), Group II (50 µg/ml,) Group III(100 µg/ml), Group IV(200 µg/ml), Group V(400 µg/ml) and Group VI(800 µg/ml) of Triphala extract-(experimental control), Group VII distilled water-(negative control).

Results : Group I and Group VI shown similar inhibitory effect on Streptococcus mitis, Group I and Group V and VI were equally effective against Streptococcus sanguis, Group I found to be more effective than all concentrations of Triphala against Actinomyces viscosus, Group I and Group V and VI were equally effective against Actinomyces naeslundii.

Conclusion : It was concluded that there was significant difference between the triphala and chlorhexidine mouthwash.

Introduction

Human dental plaque is one of the ecosystems in which microorganism was first observed[1]. Dental plaque refers to the aggregates of bacterial cell embedded in a polysaccharide and protein matrix which adheres to the teeth by characteristic bacteria grouped as early colonizers and late colonizers. Early colonizers like *Streptococcus mitis, Streptococcus oralis, Actinomyces species*, and *Neisseria species* are of great importance in the succession stages of biofilm formation because after adhering to the tooth surface,

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they provide attachment substrates for the subsequent colonizers and ultimately influence the succeeding stages of biofilm formation and its overall effect on the oral health of the host.[2,3]

Patients and dental professionals must act as co-therapists if long-term perseverance for successful plaque control is to be achieved. In order to reduce this high necessity of very demanding commitment, many chemicals have been proposed to control supragingival dental plaque, and the subject has been reviewed on many occasions. Chlorhexidine, a cationic bisguanide, has been established as a most effective chemical plaque control compound with a broad spectrum antiseptic with pronounced antimicrobial effect on gram positive and negative bacteria, yeast, dermatophytes and some lipophilic viruses and also potent anti-plaque agent but it has been reported to originate some reversible local side effects such as, staining on teeth and tongue, perturbation of the taste, oral mucosal erosions and enhanced supragingival calculus deposition. Several anti-plaque agents are available in the market, however, many undesirable side effects associated with these agents stimulated the search for alternative agents. Hence, in recent years, the focus has shifted on plants and plant products used in folk dental practice or presumed in Unani, Homeopathic or Ayurvedic remedies.[3]

Triphala is an age old drug used as a first line treatment for many aliments and is used as laxative, detoxifying agent and rejuvenator. Each component of Triphala has got an array of therapeutic activity including analgesic, antipyretic, immunomodulatory, cytoprotective, antitussive, gastroprotective, antioxidant, antimicrobial, antianaphylactic, radioprotecive, antispasmodic, bronchodilatory, hypercholesterolemic, hypoglycemic, cardioprotective, and hepatoprotective activities.[4] It has proved its efficacy as root canal irrigant and mouth rinse. In dentistry, the antimicrobial activity of Triphala has been tested against *Streptococcus mutans*[3], *Lactobacillus*[5] and *Enterococcus faecalis*[6] only.

Literature available is still deficient in studies regarding the effect of Triphala on other microorganisms of dental plaque. Hence, the aim of this study is to assess antibacterial effect of Triphala on early colonizers of dental plaque biofilm.

Aims and Objectives

To evaluate the antibacterial activity of Triphala on following early colonizers of dental plaque

Streptococcus sanguis, Streptococcus mitis, Actinomyces naeslundii, Actinomyces viscosus

To assess the most effective concentration of Triphala for its antimicrobial activity.

To evaluate the antibacterial activity of 0.2% Chlorhexidine on early colonizers of dental plaque.

To compare the antibacterial activity of 0.2% Chlorhexidine and five different concentrations of Triphala herbal extract on early colonizers of dental plaque.

Materials and Method

An in vitro study was conducted at the Bharati Vidyapeeth Dental College And Hospital, Pune, Maharashtra. India, to evaluate antibacterial effect of Triphala herbal extract on early colonizers of dental plaque biofilm after obtaining approval from institutional ethical committee. A total of 30 children of both the sexes aged between 8 – 12 years were selected. Before the commencement of this study, an informed consent from the participating subjects and their parents was obtained.

Solutions used:

Aqueous Triphala extract (50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml)

0.2% chlorhexidine (Hexidine, ICPA, India)

Distilled water

Supragingival plaque sample collection

All biofilm samples were collected by one investigator from 30 subjects who had a caries free buccal / labial tooth surface Biofilm were collected 2 hours after pumicing with no sampling in between. Subjects were advised to refrain from eating, drinking (except water) and brushing during the biofilm formation phase.

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Biofilm formed was collected with periodontal scalers by applying mild pressure on the collection area and was transferred into thioglycollate broth transport media.

Preparation of triphala extract

Powders of fruits of three plants, *Terminalia chebula, Terminalia belerica and Emblica officinalis* were added in 1:1:1 proportion by weight (50 gm each weighed using electronic weighing scale) to 16 parts (2400 ml) of water. The solution was filtered to remove any suspended impurities using muslin cloth and the filtrate obtained was boiled at 50° C till all the water content of the filtrate was evaporated & 100% aqueous extract of Triphala was obtained in solid form[7].

To obtain a concentration of 50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml, 0.5 mg, 1 mg, 2 mg, 4 mg and 8 mg of solid extract was dissolved in 10 ml of distilled water respectively.

Microbial analysis

Streptococcus species and Actinomyces species, respective were incubated in McIntosh Field's anaerobic jar and the anaerobic atmosphere was created. The plates were inoculated for 48-72 hours. The micro-organisms were identified based on the colony characters such as size, shape, hemolysis and pigmentation were noted.

Agar well diffusion assay

After isolation colonies of individual micro-organism were taken and inoculated in Brain Heart Infusion agar (Muller Hinton Medium) by lawn culture method[8]. Seven wells of 8mm diameter were punched with a sterile cork borer aseptically and 50µl of each dilution of Triphala, 0.2% Chlorhexidine and distilled water were added to wells and incubated at 37°C for 24 hours. Next day the antibacterial activity was measured as the zone of inhibition diameters (in mm) using ruler.(Fig.1)

Fig. 1

Results and Statistical analysis

The anti-bacterial activity of three test agents against early colonizers of dental biofilm (*S. sanguis, S. sanguis, A. viscosus, A. naeslundii*) was assessed in the following groups:

Positive control (Group I): 0.2% Chlorhexidine.

Experimental group: Aqueous extract of Triphala with five different concentrations as **50µg/ml (Group II), 100µg/ml (Group III), 200µg/ml (Group IV), 400µg/ml (Group V)** and **800µg/ml (Group VI).**

Negative control (Group VII): Distilled water.

The results were statistically analyzed using one way ANOVA for multiple group comparisons and Tukey's Post Hoc analysis for intergroup comparisons. Distilled water was inactive against all test micro-organisms, thus this group was not subjected to statistical analysis.

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Table I, Graph I: Mean and standard deviation of the zones of inhibition (in mm) for *Streptococcus mitis* with five different concentrations of Triphala, 0.2% CHX and Distilled water (Fig.18)

Streptococcus mitis								
Groups		N	Mean	SD	Minimum (in mm)	Maximum (in mm)	F	Sig.* (p value)
I	0.2% CHX	30	22.77	1.96	19	26		
II	50 µg/ml	30	8.07	1.31	5	10		
III	100 µg/ml	30	10.50	1.20	8	13		
IV	200 µg/ml	30	12.50	1.85	10	17	433.21	< 0.001
V	400 µg/ml	30	17.10	1.35	15	20		
VI	800 µg/ml	30	21.07	1.53	18	24		
VII	Distilled water	30	-	-	-	-		

N = number of samples, SD = Standard Deviation, F – Index of ANOVA*, p value < 0.05 – statistically significant

By using ANOVA test p-value < 0.05 therefore there is significant difference between mean zone of inhibition count with respect to treatment group for streprococcus mitis.

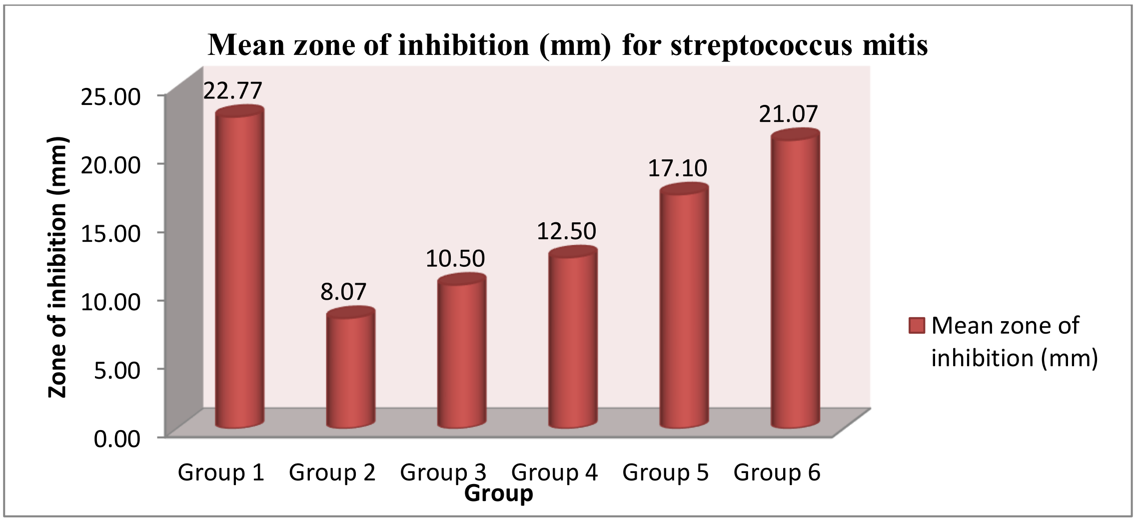

Table II, Graph II: Mean and standard deviation of the zones of inhibition (in mm) for *Streptococcus sanguis* with five different concentrations of Triphala, 0.2% CHX and Distilled water (Fig.19)

Streptococcus sanguis								
Groups		N	Mean	SD	Minimum (in mm)	Maximum (in mm)	F	Sig.* (p value)
I	0.2% CHX	30	21.2	1.85	18	26	320.04	< 0.001

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II	50 µg/ml	30	8.07	1.82	5	12		
III	100 µg/ml	30	10.47	1.66	7	14		
IV	200 µg/ml	30	12.83	1.58	10	17		
V	400 µg/ml	30	17.20	1.27	15	21		
VI	800 µg/ml	30	20.67	1.79	18	24		
VII	Distilled water	30	-	-	-	-		

N = number of samples, SD = Standard Deviation, F – Index of ANOVA*, p value < 0.05 – Statistically significant By using ANOVA test p-value < 0.05 therefore there is significant difference between mean zone of inhibition count with respect to treatment group for streprococcus sanguis

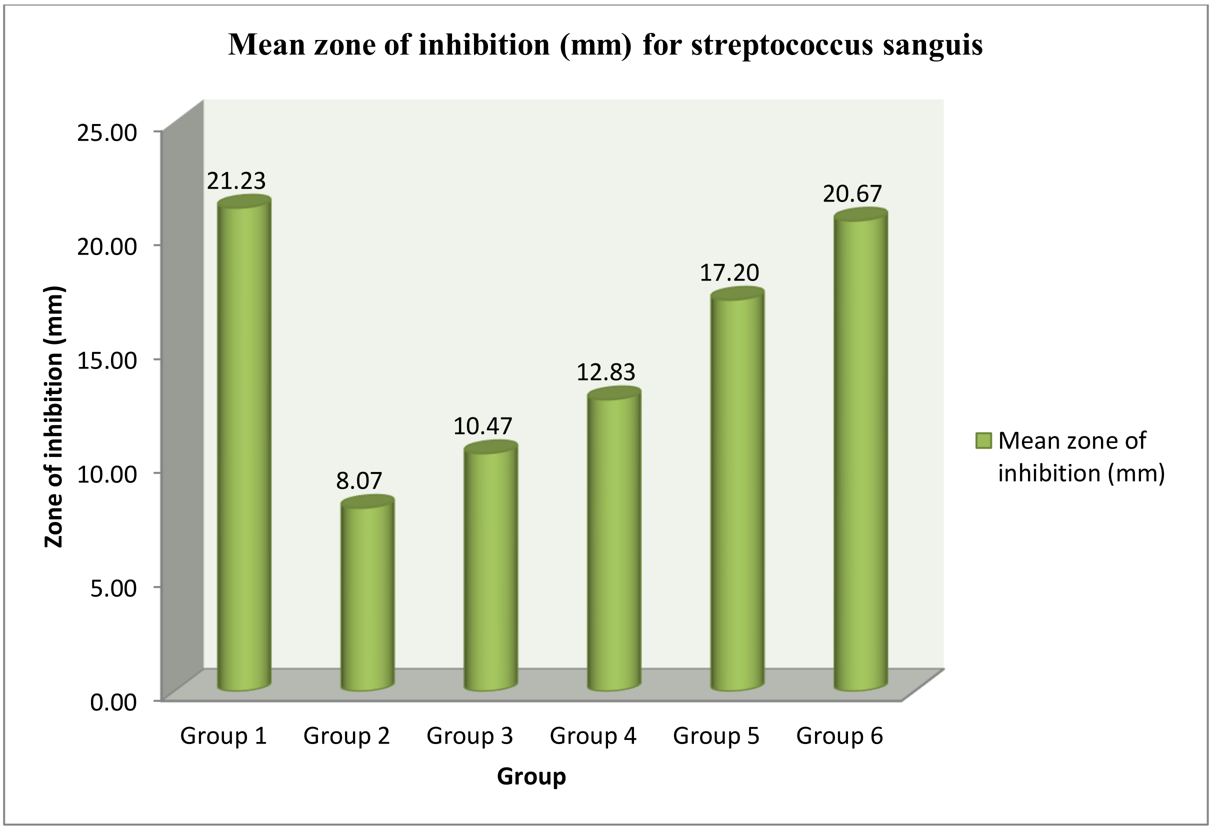

Table III, Graph III: Mean and standard deviation of the zones of inhibition (in mm) for *Actinomyces viscosus* with five different concentrations of Triphala, 0.2% CHX and Distilled water (Fig.20)

Actinomyces viscosus								
Groups		N	Mean	SD	Minimum	Maximum	F	Sig.* (p value)
I	0.2% CHX	30	21.57	1.43	19	24	374.11	< 0.001

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II	50 µg/ml	30	7.20	1.45	5	10		
III	100 µg/ml	30	10.30	1.24	8	13		
IV	200 µg/ml	30	13.80	1.86	10	17		
V	400 µg/ml	30	17.60	1.83	15	21		
VI	800 µg/ml	30	20.40	1.79	17	24		
VII	Distilled water	30	-	-	-	-		

N = number of samples, SD = Standard Deviation, F – Index of ANOVA*, p value < 0.05 – statistically significant By using ANOVA test p-value < 0.05 therefore there is significant difference between mean zone of inhibition count with respect to treatment group for Actinomyces Viscosus.

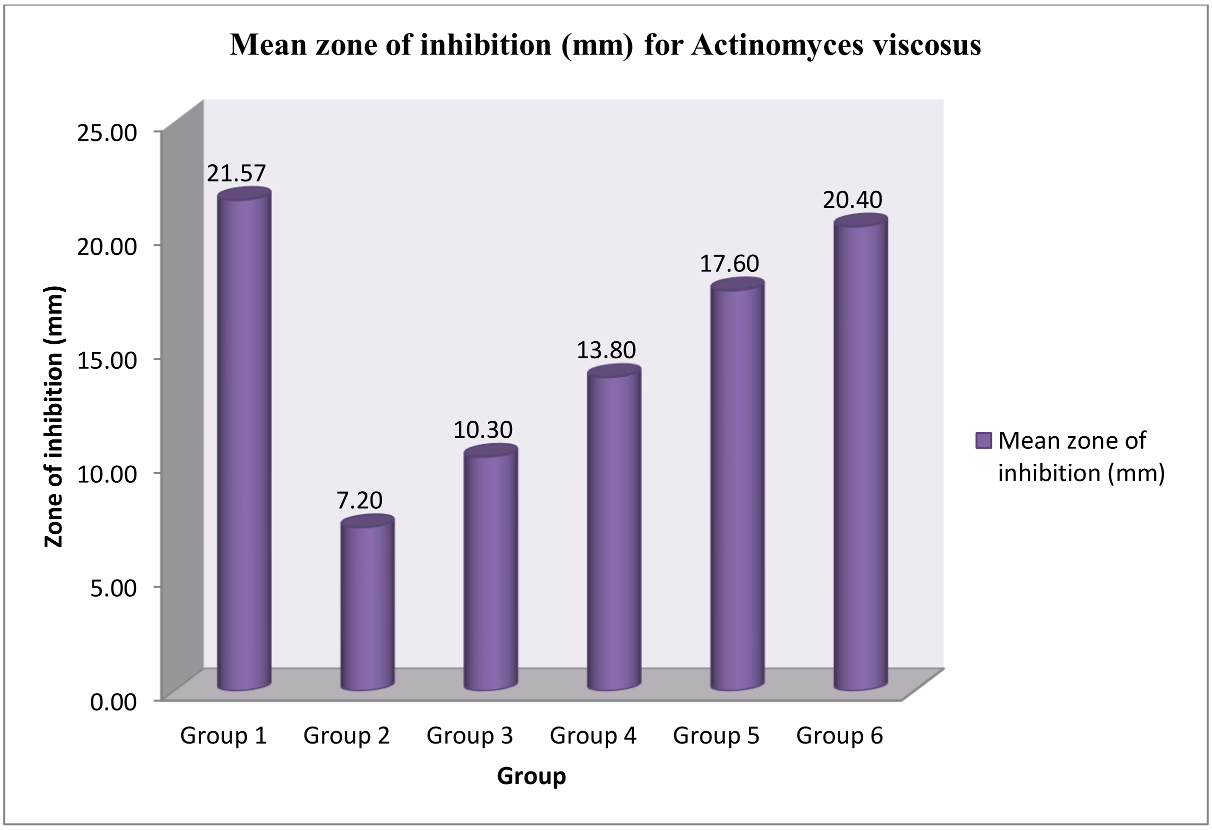

Table IV, Graph IV : Mean and standard deviation of the zones of inhibition (in mm) for *Actinomyces naeslundii* with five different concentrations of Triphala, 0.2% CHX and Distilled water (Fig.21, Fig.22)

Actinomyces naeslundii								
Groups		N	Mean	SD	Minimum	Maximum	F	Sig.* (p value)
I	0.2% CHX	30	22.03	1.67	19	25	444.01	< 0.001
II	50 µg/ml	30	8.13	1.46	5	11		

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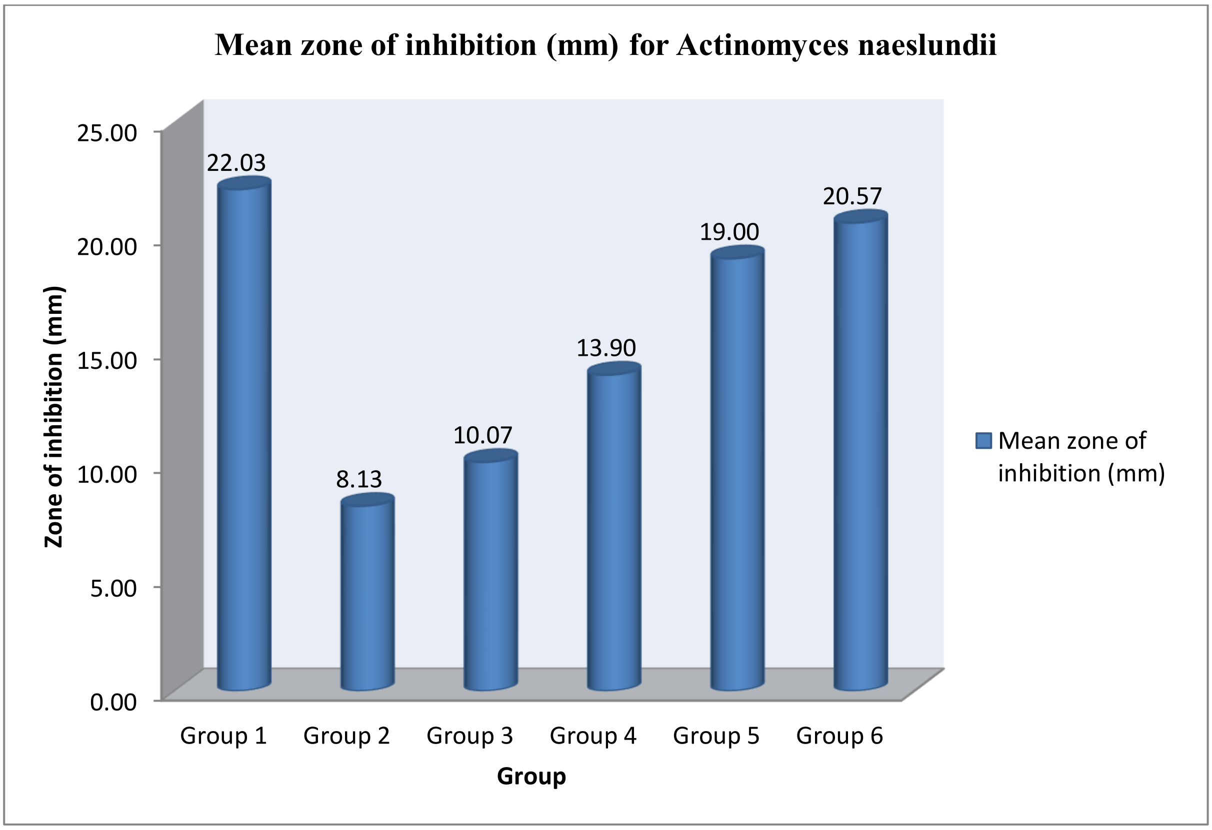
III	100 µg/ml	30	10.07	1.23	8	13		
IV	200 µg/ml	30	13.90	1.60	11	16		
V	400 µg/ml	30	19.00	1.53	16	22		
VI	800 µg/ml	30	20.57	1.48	17	23		
VII	Distilled water	30	-	-	-	-		

N = number of samples, SD = Standard Deviation, F – Index of ANOVA*, p value < 0.05 – statistically significant By using ANOVA test p-value < 0.05 therefore there is significant difference between mean zone of inhibition count with respect to treatment group for Actinomyces naeslundii.



Discussion

Natural compounds contained in various herbs can act in a synergetic manner within the human body, and can provide unique therapeutic properties with minimum or no undesirable side effects. One such herbal cocktail is Triphala, the herbal product of equal proportion of dry powder of *Terminalia chebula, Terminalia belerica* and *Emblica officinalis*. Among various therapeutic effects of Triphala, its anti-oxidant, anti-inflammatory and anti-microbial activities may prove to be of great importance in prevention and treatment of gingival and periodontal diseases. Triphala and its individual components have been reported to be effective in prevention and treatment of scorbutic gingivitis, peri-coronitis, ANUG, focal reversible pulpitis, periodontitis, dental caries, oral ulcers, and in root canal infection[9].

Thus the present study was conducted to evaluate the antibacterial activity of five different concentrations of Triphala herbal extract (50µg/ml, 100µg/ml, 200µg/ml, 400µg/ml, and 800µg/ml), 0.2% Chlorhexidine (Positive control) and distilled water (Negative control) on four early colonizers of dental biofilm, *S. sanguis, S. sanguis, A. viscosus, A. naeslundii.*

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In this study distilled water was taken as negative control to which all four organisms were found to be resistant as there was no inhibition zone around wells containing distilled water.

Streptococcus mitis:

0.2% CHX and 800 µg/ml aqueous Triphala extract have shown similar inhibitory effect on *Streptococcus mitis.* Similar results were observed in studies conducted by Yeun et al (1998)[10], Haffajee et al (2008)[11] which demonstrated inhibitory effect of CHX on growth of *Streptococcus mitis*.

0.2% CHX, 400 µg/ml and 800 µg/ml aqueous Triphala extract were equally effective against *S. sanguis,*. Similar observations were made by Newman et al (1979)[12], demonstrating inhibitory effect of different concentrations of CHX on growth of *S. sanguis.*

Actinomyces viscosus:

0.2% CHX was more effective than all concentrations of Triphala aqueous extract against *Actinomyces viscosus* in all the samples while other concentrations of Triphala aqueous extract were less effective as compared to 0.2% CHX. and Wang et al (2007)[13] similarly observed antibacterial activity of 0.02% CHX against *A. viscosus.*

Actinomyces naeslundii:

0.2% CHX, 400 µg/ml and 800 µg/ml aqueous Triphala extract were equally effective against *Actinomyces naeslundii.*

Since there are no comparative studies exist in periodontal literature in children, assessing the antimicrobial activity of Triphala aqueous extract on early colonizers of dental biofilm assessed in this study, the results of this could not be correlated.

While comparing CHX and Triphala other factors such as substantivity, side effects, cellular toxicity and cost must be taken into consideration. The major advantage of chlorhexidine over most other compounds lies in its substantivity. It binds to soft and hard tissues in the mouth, enabling it to act over a long period after use of a formulation. Bacterial counts in saliva consistently drop to between 10 and 20% of baseline after single rinses and remain at this level for at least 7 hours and probably more than 12 hours. One of the three components of Triphala, Terminalia chebula showed to be effective for up to 3 hours after rinsing[14]. On the contrary, there is no data available today assessing substantivity of Triphala, thus this aspect needs to be explored.

CHX has reported many local and systemic side effects on long term use including staining of teeth, taste perturbation, oral mucosal erosions, parotid swelling and enhanced rate of calculus formation, none of which were reported from any study assessing Triphala as a mouthwash.

Sluss[15] stated the problem, “Antisepsis, whatever the agent employed, must always be regulated by two cardinal principles: the maximum germicidal efficiency; minimum injury to the tissues”. The ability of chlorhexidine to non-selectively kill oral microbiota makes it an excellent agent to indiscriminately affect mammalian cells. In fact; the effects of chlorhexidine on a variety of mammalian cells have shown this drug to be a toxic agent at doses similar to or below those introduced into the oral cavity. Chlorhexidine application directly to surgical wounds in the oral cavity can delay and alter wound healing. On the other hand, Triphala has shown its beneficial effect on synthesis and proliferation of fibroblasts and thus improved healing of infected dermal wound[61] which can also be useful in healing of periodontal lesions.

As a mouthwash, this research shows that Triphala is equally effective to prescription mouthwashes containing Chlorhexidine in reducing and preventing plaque, gingivitis, streptococcus species, Actinomyces species (bacteria known to cause tooth decay, gingival and periodontal diseases) Thus, Triphala may prove to be a promising alternative antimicrobial agent.

Conclusion

In this study, among all tested concentrations of Triphala aqueous extract 400µg/ml and 800µg/ml were found to be equally effective against *Streptococcus mitis, Streptococcus sanguis,* and *Actinomyces naeslundii* while 0.2% Chlorhexidine was better than all tested concentrations of Triphala against *Actinomyces viscosus and naeslundii.* Triphala possesses many different therapeutic properties such as anti-inflammatory and anti-oxidant activity.

Thus Triphala may prove to be a very useful alternative agent in prevention and treatment of gingival and periodontal diseases and tooth decay

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Triphala mouthwash gives us yet another way to keep our daily routine natural. Good for your body and for the environment.

Acknowledgement

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