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Antimicrobial Effects of Streblus Asper Leaf Extract: A Randomized Controlled Clinical Trial

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Abstract

Aim: This study will investigate the antibacterial effects of streblus asper leaf extract on plaque formation, gingival health and reduction in colony forming units of *Streptococcus mutans and Actinomycetemcomitans.*

Materials and Methods: A Randomized Controlled Trial was conducted among 14-18 years old school students of Bhubaneswar city to determine the antibacterial effects of streblus asper leaf extract on plaque formation, gingival health and reduction in colony forming units of *Streptococcus mutans and Actinomycetemcomitans.*

Results: The baseline mean of the plaque index was found to be 2.42 in the chlorohexinde group, 1.25 in the placebo group, 2.22 streblus asper alcoholic extract group and 2.31 in streblus asper aqueous extract group and the baseline mean of the gingival index was found to be 2.12 in the chlorohexidine group, 2.23 in the streblus asper alcoholic extract group, and 2.13 in the streblus asper aqueous extract group was found to be statistically significant p ≥0.001.

Conclusion: The results of the present study have shown that SAE when used in a mouthrinse has a clinically measurable effect on gingival health without significant effect on plaque growth. Additional usage of SAE mouthrinse to routine mouth cleaning may enhance the protective value to oral hygiene.

Keywords: Mouthrinse; Plaque; *Streptococcus mutans; Actinomycetemcomitans*

Introduction

Oral health is very important to the appearance as well as the sense of well being. There is various emerging evidence that has shown a strong link between the effects of oral health on general health. According to WHO (World Health Organization). Majority of population in the society has been affected by gingival and periodontal diseases and the main etiology behind these diseases is plaque [1]. Periodontal diseases are chronic inflammatory conditions characterized by loss of connective tissue, alveolar bone resorption and formation of periodontal pockets as a result of the complex interaction between pathogenic bacteria and the host's immune response [2]. There are various mechanical and chemical plaque control aids being used to control plaque. Mechanical methods include tooth brushing interproximal cleaning using dental floss or interproximal brushes. Chemical methods include the use of dentrifice, mouthwashes [3].

Chemical mouthwash containing chlorohexidine is the most used and is gold standard in antimicrobial efficacy [4]. These chemical mouthwashes have a lot of disadvantage like discoloration of teeth, dryness of mouth, erosion of enamel etc [5]. Whereas herbal mouthwashes provides a viable alternative as they are alcohol free chemical free which will not cause any kind of side effects or disadvantages for the oral health. In the present study we have used streblus asper leaf extract. Streblus asper belongs to the family of Moraceae which is a small tree mostly found in tropical countries like India, Sri Lanka, Malaysia and Thailand. It is known by various name eg Bar-inka, Berrikka, Rudi, Sheora, Koi it is also known as toothbrush tree [6]. In India it is known by its several vernacular names, the most commonly used ones being Shakhotaka (Sanskrit), Siora (Hindi), Sheora (Bengali) and Piray (Tamil). It is used traditionally in leprosy, piles, diarrhea, dysentery, elephantiasis and cancer. It is a rigid

shrub or gnarled tree; branchlets tomentose or pubescent [6]. In different biological activities of S. asper in various in vitro and in vivo test models. Different parts of this plant have been found to exhibit cardiotonic, antifilarial, anticancer, antimicrobial, anti allergic and antimalarial activities [7].

Studies demonstrated the antimicrobial activity of *S asper* leaf extract upon various microorganisms involving oral and naso pharyngeal infections, especially *S. mutans*. The extract possess a selective bactericidal activity towards streptococcus especially to *S. mutans* which has shown to be strongly associated with dental caries and it has also shown antibacterial activity towards anaerobic bacteria such as C. albicansn [7]. As there is very less research being carried out on streblus asper leaf extract and its effect on oral health. Therefore this study was carried out to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of streblus asper aqueous extract and alcoholic extract upon ATCC strain of *Streptococuss mutans* and *Actinomycetemcomitans*. To compare the antimicrobial activity of streblus asper mouthrinse with chlorhexidine on salivary *Streptococcus mutans* and *Actinomycetecomitans*.

Materials and Methods

Study Population

A Randomized Controlled Trial was conducted among 14-18 years old school students of Bhubaneswar city to determine the antibacterial effects of streblus asper leaf extract on plaque formation, gingival health and reduction in colony forming units of *Streptococcus Mutans* and *Actinomycetemcomitans*.

Sample Size Determination

Sample size estimation was done basing on the empirical data by using G Power software (version 3.0). A minimum total sample size of 76 (19 per group) was derived.

Brief Profile of the Study Area

Bhubaneswar, is the capital of the Indian state **of** Odisha. It is the largest city in Odisha and is a centre of economic and cultural importance in Eastern India. As per the 2011 census of India, Bhubaneswar had a population of 837,737, while the metropolitan area had a population of 881,988. Effective male literacy was 95.69 per cent, while female literacy was 90.26 per cent. About 75,237 were under six. Bhubaneswar's literacy rate is 93.15 per cent—significantly higher than the national average of 74.04 per cent.

Study Population

Subjects were selected from an institutionalized school in Bhubaneswar. The dietary pattern and the socioeconomic strata were thus, standardized.

Procurement of S. Asper Leaf Extract

Leaves of S. asper were locally collected from Jagatsingpur Odisha province during the month of September 2018.

Inclusion Criteria

i. Students, without any oral appliances and who will not be having any relevant medical history.

ii. All participants will be selected based on having moderate gingival inflammation (Gingival index will be taken Score range is from 0 to 3 if the participants fall under the range of score 2 will be selected)

Exclusion Criteria

i. Students not willing to participate.

ii. Students who shall have not received systemic antibiotics in the preceding three months and did not receive such pharmacotherapy during the study.

iii. Students with orthodontic braces and fixed partial dentures will be excluded.

Preparation of Streblus Asper Leaf Alcoholic Extract (SAE)

The leaves were washed, air-dried and pulverized. The dried-pulverized Streblus asper leaves were extracted by the method previously described. Total grams of powder obtained from 1.5kgs of leave was 534gm and soaked in 3750 litre of distilled water and 3750 ethanol for seven days and it was stirred occasionally. After seven days of occasional stirring of the extract. Then tne extract was passed through several layers of cheese cloth to get the final sticky material. Approximately 75 g of dark brown sticky material was obtained from 1500 g of the dried-pulverized leaves. The material was dissolved in distilled water at 250 mg/ml, centrifuged at 10,000 rpm (9410 x g) at 4oC for 20 min and passed through a 0.2 µm filter. The filtrate was used as the starting material for subsequent studies. After preparation of the leave extract the minimum bactericidal concentration, minimum inhibitory concentration along with the toxicity and the antimicrobial efficacy will be checked in the laboratory.

Preparation of Streblus Asper Leaf Aqueous Extract (SAE)

The leaves were washed, air-dried and pulverized. The dried-pulverized Streblus asper leaves were extracted by the method previously described. One litre of distilled water was mixed with 100 gms of powder. Then the solution was passed through several layers of cheese cloth and then a clear aqueous extract was obtained.

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Detection of MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration)

Antibacterial effects of SAE (*Streblus Asper Leaf Extract*) on ATCC (70717) actinomycetemcomitans was determined by the disk diffusion method. Plates containing Blood agar were seeded with the bacterial suspensions. Seeding was done using sterile swabs that were brushed across the agar surfaces. Paper discs (6 mm in diameter) soaked with 15 µL of 250 and 500 mg/mL SAE, 5% NaOCl. Then the plates were placed into anaerobic jars and sealed. Anaerobic conditions were maintained using the Gas Pack System After 1 week of incubation at 37°C, the zones of inhibition were measured across the diameter with a transparent ruler and recorded using the following criteria (- = No zone of inhibition; + = Zone of inhibition > 6-8 mm; ++ = Zone of inhibition > 8 mm). The tests were performed in triplicate for each bacterial strain.

Effects of SAE (Streblus Asper Leaf Extract) on ATCC (70718) *Streptococcus mutans* was determined by double dilution technique. In this technique, *S. mutans* ATCC (70718) was cultured and adjusted with Mueller–Hinton broth to give a final absorbance at 600 nm=0.1 or :100×106 CFU ml−1 . Fifty microliters of bacterial suspension was mixed with 50 ml of *S. asper* extract which was two-fold serially diluted with Mueller–Hinton broth in a microtitre plate. The bacteria were cultured for 18–20 h at 37°C under 5% CO_2. Viability of the bacteria in each well was examined by sub culturing in blood agar plates. The lowest concentration of the S. asper extract which inhibited the growth of bacteria was recorded as the Minimum Bactericidal Concentration (MBC)

Experimental Design

It was a 21-day randomized control clinical trial where the subjects were divided into four groups. Group A chlorohexidine mouth rinse (0.2%), group B was given placebo group C was given Streblus asper alcoholic extract and group D was given *Streblus asper* aqueous solution .

Clinical Trial: Rinsing procedure and saliva collection

All the children participating in the present study were instructed their teeth on the day of sampling and to follow their daily diet. The children were randomly assigned to four different groups. Group A chlorohexidine mouth rinse (0.2%) , group B was given placebo group C was given *Streblus asper* alcoholic extract and group D was given *Streblus asper* aqueous solution. All the participants were provided with a sterile sample collector. At zero hour saliva samples of all participants belonging to all the four groups were collected and then the participants belonging to each group were given the above mentioned mouthwashes and asked to rinse for one minute. After which the unstimulated saliva samples were collected from all the participants of four different groups at 2 mins, 30 mins, one hour and two hours respectively Figure 1.

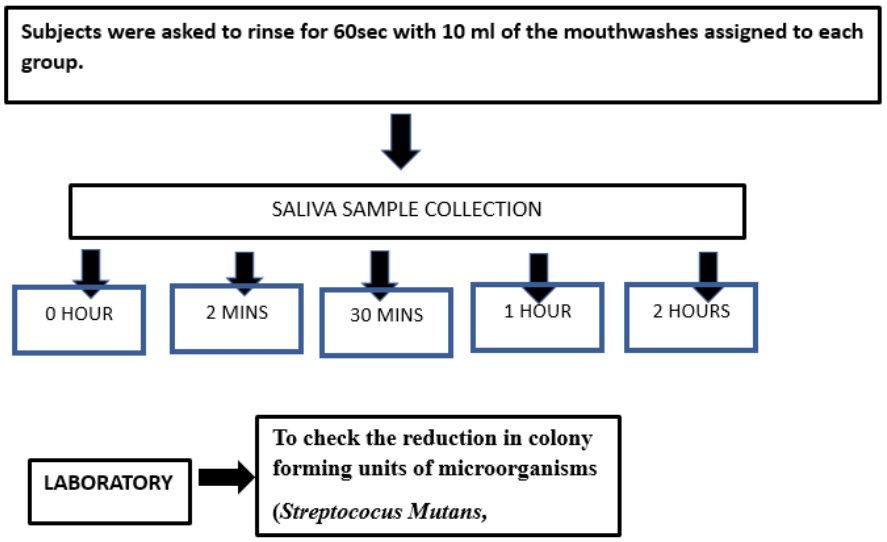

Figure 1: Saliva Sample Collection.

Antimicrobial Assay

After the saliva samples were collected then it was transferred to the laboratory within four hours once the saliva samples reached the laboratory then they were cultured on blood agar plates which was divided into five different time zones 0 hour, 2 mins, 30 mins, one hour and two hour respectively. A total of 40 blood agar plates were used. Plaque samples were pooled, streaked on blood agar plate, incubated at 37°C for 24-28 hrs. Microorganisms

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were stained with Gram staining and were detected under high power microscope were used to identify specific microorganisms. The blood agar plates were prepared separately for aerobic and anaerobic bacteria for aerobic bacteria it was incubated at 37°C in a candle jar for 24-28 hours and for anaerobic bacteria it was incubated inside a glass with a gas pouch at 37 °C for 24-28 hours. After 24-28 hours the colony forming units were checked for both aerobic and anaerobic bacteria Figure 2.

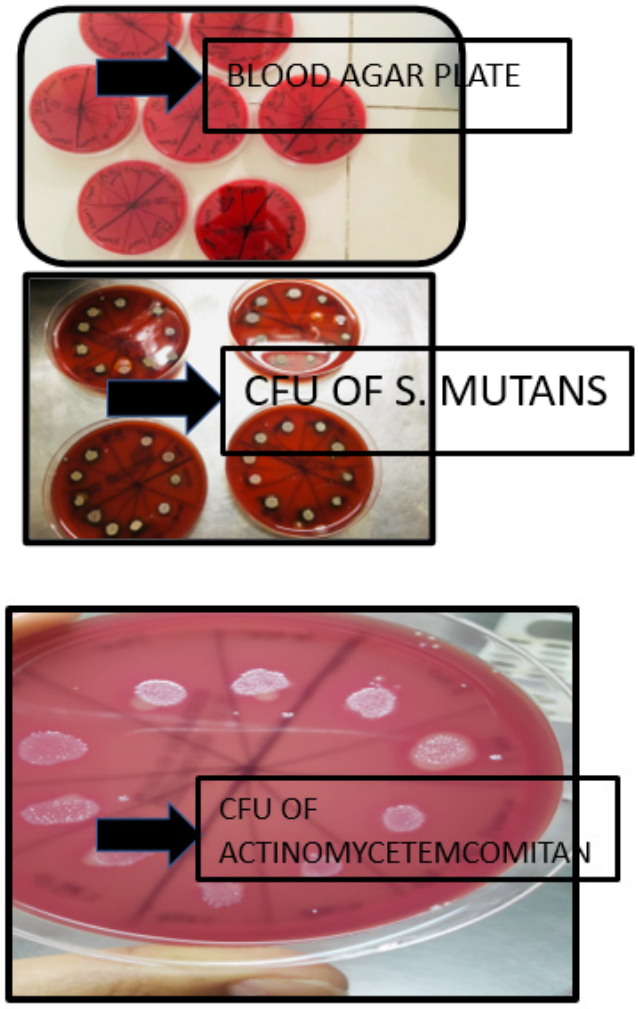

Figure 2: The blood agar plates were prepared separately for aerobic and anaerobic bacteria for aerobic bacteria it was incubated at 37□ in a candle jar for 24-28 hours and for anaerobic bacteria it was incubated inside a glass with a gas pouch at 37□ for 24-28 hours. After 24-28 hours the colony forming units were checked for both aerobic and anaerobic bacteria.

Results

The mean comparison of age among all the groups was found to be 14.31 in the chlorohexidine group followed by 14.36 in the placebo, 14.89 in streblus asper alcoholic group, 14.21 in streblus asper aqueous extract. Among all the groups the mean age was found to be statistically significant p ≥0.001 (Table 1). The mean comparison of plaque index baseline among all the four groups was found to be 2.42 in the chlorohexidine group, 1.25 in the placebo group, 2.22 in the streblus asper alcoholic group, 2.31 in the streblus asper aqueous group was found to be statistically significant p ≥0.001. The mean compression of plaque index post 7 days was found to be 1.23 in the chlorohexidine group, 1.73 in the placebo group, 1.06 in the streblus asper alcoholic extract group, 1.13 in the streblus asper aqueous extract group it was found to be statistically significant p ≥0.001. The mean comparison of plaque index post 14 days was found to be 1.09 in the chlorohexidine group, 1.65 in the placebo group, 1.19 in the streblus asper alcoholic extract group, .936 in the streblus asper aqueous extract group it was found to be statistically significant p ≥ 0.001 (Table 2).

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Table 1: The baseline mean of the plaque index was found to be 2.42 in the chlorohexinde group, 1.25 in the placebo group, 2.22 streblus asper alcoholic extract group and 2.31 in streblus asper aqueous extract group and the baseline mean of the gingival index was found to be 2.12 in the chlorohexidine group, 2.23 in the streblus asper alcoholic extract group, and 2.13 in the streblus asper aqueous extract group was found to be statistically significant p ≥0.001.

	Age	Plaque Index Baseline	Plaque Index Post 7 Days	Plaque Index Post 14 Days	Plaque Index Post 21 Days	Gingival Index Baseline	Gingival Index Post 21 Days
N	76	76	76	76	76	76	76
Mean	14.44	2.051	1.29	1.21	0.97	1.91	1.12
Standard Deviation	0.719	0.56	0.407	0.386	0.658	0.482	0.399

Table 2: The mean comparison of plaque index post 21 days was found to be 1.19 in the chlorohexidine group, 1.78 in the placebo group, .365in the streblus asper alcoholic extract group, .555 in the streblus asper aqueous extract group it was found to be statistically significant p ≥0.001.

Chlorohexidine	Age	Plaque Index Baseline	Plaque Index Post 7 Days	Plaque Index Post 14 Days	Plaque Index Post 21 Days	Gingival Index Baseline	Gingival Index Post 21 Days
N	19	19	19	19	19	19	19
Mean	14.3	2.42	1.23	1.09	1.19	2.12	1.12
Standard Deviation	0.477	0.232	0.294	0.134	0.284	0.159	0.179

The mean comparison of gingival index baseline among all the four groups was found to be 2.12 in the chlorohexidine group, 1.17 in the placebo group, 2.23 in the streblus asper alcoholic group, 2.13 in the streblus asper aqueous group was found to be statistically significant p ≥0.001 (Table 3). Comparison of mean scores of plaque index from baseline till 21 days among the chlorohexidine group. The mean scores of plaque index at baseline was found to be 2.42 post 7 days it was found to be 1.23 which was statistically significant p≥ 0.001. Post 14 days the mean scores of plaque index was found to be 1.09 which was non-significant when compared with post 7 days (Table 4). Comparison of mean scores of gingival index from baseline and 21 days among the Chlorohexidine group (Table 5).

Table 3: The mean comparison of gingival index post 21 days was found to be 1.12 in the chlorohexidine group, 1.55 in the placebo group, .862 in the streblus asper alcoholic extract group, .967 in the streblus asper aqueous extract group it was found to be statistically significant p ≥0.001.

Placebo	Age	Plaque Index Baseline	Plaque Index Post 7 Days	Plaque Index Post 14 Days	Plaque Index Post 21 Days	Gingival Index Baseline	Gingival Index Post 21 Days
N	19	19	19	19	19	19	19
Mean	14.3	1.25	1.73	1.65	1.78	1.17	1.55
Standard Deviation	0.683	0.504	0.498	0.488	0.516	0.338	0.463

Table 4: Post 21 days the mean scores of the plaque index was found to be 1.19 which was non-significant when compared post 14 days.

Streblus Asper Alcoholic Solution	Age	Plaque Index Baseline	Plaque Index Post 7 Days	Plaque Index Post 14 Days	Plaque Index Post 21 Days	Gingival Index Baseline	Gingival Index Post 21 Days
N	19	19	19	19	19	19	19
Mean	14.8	2.22	1.06	1.19	0.365	2.23	0.862
Standard Deviation	0.994	0.085	0.141	0.076	0.221	0.116	0.213

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Table 5: The mean scores of gingival index at baseline was found to be 2.12 and post 21 days it was found to be 1.12 which was statistically significant p≥ 0.001.

Streblus Asper Aqueous Solution	Age	Plaque Index Baseline	Plaque Index Post 7 Days	Plaque Index Post 14 Days	Plaque Index Post 21 Days	Gingival Index Baseline	Gingival Index Post 21 Days
N	19	19	19	19	19	19	19
Mean	14.2	2.31	1.13	0.936	0.555	2.13	0.967
Standard Deviation	0.418	0.274	0.199	0.233	0.31	0.17	0.28

Discussion

Streblus asper Lour (Family: Moraceae) is a small tree which is indigenous to tropical countries such as India, Sri Lanka, Malaysia, the Philippines and Thailand. It is known by various names, e.g. Barinka, Berrikka, Rudi, Sheora, Koi, Siamese rough bush and tooth brush tree. In India it is known by its several vernacular names, the most commonly used ones being Shakhotaka (Sanskrit), Siora (Hindi), Sheora (Bengali) and Piray (Tamil). It is used traditionally in leprosy, piles, diarrhea, dysentery, elephantiasis and cancer. It is a rigid shrub or gnarled tree; branchlets tomentose or pubescent [8]. In Odisha it is found in abundance and commonly known as Sahada. The bark of the tree has been used for various tooth related problem like toothache, gingivitis and the bark of the tree is used as a toothbrush in Odisha since primitive time other than toothache problems it has also several medicinal properties.

Chlorhexidine

Chlorhexidine was developed in the late 1940s as a result of a search for antiviral agents. It was found that chlorhexidine does not possess antiviral activity but instead it possesses antibacterial activity. The use of chlorhexidine was begun as a general disinfectant with a broad antimicrobial spectrum. Its antimicrobial spectrum includes most of the microbials such as gram positive and gram-negative organism including bacterial spores, lipophilic viruses, yeasts and dermatophytes [9].

Chlorhexidine as an Antiplaque Agent

Several in vivo and in vitro studies proved efficacy of 0.2% chlorohexidine as an antiplaque agent. Effect of chlorohexidine on plaque inhibition is dose dependent, the dose usually ranges in the concentration of 0.03 to 0.2% volume, frequency and concentration are important in determining the clinical response. The optimum dose of chlorohexidine as a mouth rinse is generally considered to be 20 mg twice daily, similar levels of plaque inhibition can be achieved with larger volumes of lower concentrations [10].

Streblus Asper leaf extract as an anti plaque agent

There have been many studies conducted which has shown that the antimicrobial activity of S. asper leaf extract upon various microorganisms involving oral and nasopharyngeal infections, specially on S. mutans. Bactericidal activity was found in the 50% ethanol (v/v) extract of S. asper leaves. The extract possessed a selective bactericidal activity towards Streptococcus mutans, which is strongly associated with dental caries [11]. S. Asper is considered to have antigingivitis and astringent effects and has been used for a long time in traditional herbal medicine to counter gingivitis and for relief of toothache [12].

Plaque Score

In the present study it was found that plaque index baseline among all the four groups was found to be 2.42 in the chlorohexidine group, 1.25 in the placebo group, 2.22 in the streblus asper alcoholic group, 2.31 in the streblus asper aqueous group was found to be statistically significant p ≥0.001 which depicted that streblus asper leaf alcoholic extract and aqueous extract both has antimicrobial effect when compared with chlorhexidine.

Gingival Score

Comparison of mean scores of gingival index from baseline and 21 days among the Placebo group. The mean scores of gingival index at baseline was found to be 1.17 and post 21 days it was found to be 1.55 which was statistically non significant

Bacteriostatic and bactericidal activities of S. asper towards Streptococci and actinomycetemcomitans

Among the *Streptococci* tested, *S. mutans* was the most susceptible flora to *S. asper* alcoholic extract. The Minimum Inhibitory Concentration (MIC) and Minimum bactericidal concentration (MBC) of *S. asper* extract towards *S. mutans* and *Actinomycetemcomitans* strain was found to be 15.62 mg/mL and did not vary with the bacterial strain tested. There was no MIC and MBC detected of *S. Asper* aqueous extract against *Streptococuss mutans* strain or *actinomycetecomitans*. Where as in the study conducted by wongkham et al the leaf extract of *S.asper* at concentration 2-100mg/ml could specifically inhibit the growth of the streptococci tested [13]. In a similar study conducted it was found that the growth inhibitory activities of *S. asper* obtained from different extractions against S.mutans were compared. The activity was found in the ethanol extracts where as no activity was found in the water extract [14].

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Effect of Streblus Asper on the CFU Counts of S. Mutans and Actinomycetemcomitans

In the present study there was a reduction in the colony forming units of *Streptococus mutans* and *A. actinomycetemcomitans* at 30 mins and one hour were seen on rinising with streblus asper alcohol extract and streblus asper aqueous extract whereas after rinsing with chlorohexidine mouthrinse it was found that the colony forming units of the *Streptococus* mutans and *A. actinomycetemcomitans* were reduced at 2 mins and 30 mins after which it was observed that the CFU counts were slowly increasing post 30 mins. In the present study it was see that the streblus asper leaf extract had a longer effect on reduction of the CFU counts of the microorganisms when compared with Chlorhexidine mouthrinse which showed a shorter duration of effect on the CFU counts of the microorganisms. The present study it was found that Streblus Asper leaf alcohol and aqueous extract both had antimicrobial properties reduced plaque formation and CFU counts of *Streptococus mutans* and *A actinomycetemcomitans* like cholorhexidine mouthrinse which is a gold standard mouth rinse.

Conclusion

The results of the present study have shown that SAE when used in a mouthrinse has a clinically measurable effect on gingival health without significant effect on plaque growth. Additional usage of SAE mouthrinse to routine mouth cleaning may enhance the protective value to oral hygiene. Since herbal extracts are natural products, the complexity of the constitution makes impossible the identification of one or other specifically active ingredient, but clearly such recipes are worthy of further attention.

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