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EVALUATION AND COMPARISON OF ANTIBACTERIAL EFFICACY OF *ALBIZIA LEBBECK* (L.) BENTH., *BAUHINIA VARIEGATA* L. AND CHLORHEXIDINE MOUTHWASHES: *IN-VITRO* STUDY

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ABSTRACT

Aim: Herbal medicines have therapeutic benefits, especially in plaque and gingivitis control. Therefore, the study aims to establish the minimum inhibitory concentration (MIC) of *Albizia lebbeck* (L.) Benth. (Siris tree), *Bauhinia variegata* Linn. (Mountain Ebony) and Chlorhexidine mouthwashes and to assess and compare the antibacterial effect of *Albizia lebbeck* (L.) Benth. mouthwash (Group I), *Bauhinia variegata* L. mouthwash (Group II) and Chlorhexidine (Group III) on plaque. Materials and Methods: The MIC of herbal mouthwashes and Chlorhexidine were determined against standard strains of bacteria involved in gingivitis. The antibacterial action of these mouthwashes on the supragingival plaque was determined and compared. Fifteen plaque samples were collected from fifteen individuals with gingivitis and were sent to the laboratory for processing. Data obtained was tabulated and subjected to statistical analysis using analysis of variance and paired t-test. Bonferroni post hoc test was used to know the difference between the pairs of mouthwashes. Analysis of covariance was also done to adjust for the baseline differences. Results: The MIC of Group I, Group II and Group III mouthwashes ranged 0.8 µg/ml-12.5 µg/ml, 0.4 µg/ml-25 µg/ml and 0.4 µg/ml-25 µg/ml, respectively, against the tested bacterial strains. Group I and Group II mouthwashes did exhibit antibacterial activity. However, the antibacterial efficacy of herbal mouthwashes exhibited significant differences when compared with Chlorhexidine. Conclusion: Group I and Group II mouthwashes showed antibacterial activity. However, these herbal mouthwashes showed less antibacterial action when compared to Group III.

Keywords: Antibacterial mouthwash, Chlorhexidine, dental plaque, oral bacteria, herbal mouthwash, *Albizia lebbeck* (L.) Benth., *Bauhinia variegata* Linn.

INTRODUCTION

Dental plaque elimination is the prime motive for maintaining periodontal health. Mechanical oral hygiene procedures could be insufficient if individuals cannot control supragingival biofilm. Therefore, chemical control of plaque is used as an adjunctive to those mechanical devices.[1]

Natural extracts have shown efficacy in oral health care.[2] However, Chlorhexidine is the gold standard for chemical plaque control due to its prolonged antimicrobial activity.[3] On a long-term basis, it exhibits side effects. Thus, in our study, we tried to introduce herbals which could be an alternative antiplaque agent.

The herbal mouthwashes chosen in this study contain *Albizia lebbeck* (L.) Benth. (Siris Tree) and *Bauhinia variegata* L. (Mountain Ebony) has shown antibacterial properties.[4,5] Therefore, in this study, we tried to establish the minimum inhibitory concentration (MIC) of *Albizia lebbeck* (L.) Benth., *Bauhinia variegata* Linn. and Chlorhexidine mouthwashes as well as assessed and compared the antibacterial effect of these herbs and Chlorhexidine on plaque samples.

MATERIALS AND METHODS

Patients visiting the Department of Periodontics were recruited in this study. Before enrolment, the participants were explained the need and design of the study. Ethical clearance was procured from the institutional ethical committee (IRB.No.2019/PG/PERIO/82).

Eligibility Criteria

Subjects who were systemically healthy having moderate to severe gingivitis according to Gingival Index (Loe & Sillness, 1963) were included in the study. The exclusion criteria were the subjects who had undergone periodontal treatment for the past three months, subjects who were already using a mouthwash, subjects with gross oral pathology, for example, tumour, cyst etc., subjects who were on antibiotic or anti-inflammatory medications for past six months, smokers, tobacco chewers, pregnant and lactating women.

Method of Study: In the study, commercially available non-alcoholic 0.2% Chlorhexidine mouthwash was used.

Herbal Mouthwash Preparation

The barks of the *Albizia lebbeck* (L.) Benth. and *Bauhinia variegata* Linn. was made into a coarse powder. One part of this powder was taken into a vessel, and 16 parts of water were added to it and boiled over medium heat. The decoction was prepared with the remaining four parts as the water evaporated. It was then filtered and made palatable by adding 2 g of edible camphor. This decoction was used for mouthwash.[6]

Microbiological Test

The antibacterial effects were determined against periodontopathogens, namely *Streptococcus mitis* (ATCC 6249), *Aggregatibacter actinomycetecomitans (*ATCC 43718), *Actinomycetes* (ATCC 15214) and *Porphyromonas gingivalis (*ATCC 33277). For the *in-vitro* testing, bacterial strains were maintained on blood agar media. Media and supplements were obtained and prepared following a manufacturer's instructions.

Determination of Minimum Inhibitory Concentration

The MIC was determined using the broth dilution method and to confirm the inhibitory concentration, each of these serial dilutions were plated on blood agar culture plates.

Procedure: Nine dilutions of each mouthwash were done with brain–heart infusion broth (BHI) for MIC. In the initial tube, 20 µl of the drug was added to the 380 µl BHI. For dilutions, 200 µl of BHI broth was added into the following nine tubes separately. Then from the initial tube, 200 µl was transferred to the first tube containing 200 µl of BHI broth. This was considered a 10-1 dilution. From the 10-1 diluted tube, 200 µl was transferred to the second tube to make a 10-2 dilution. The serial dilution was repeated up to 10-9 dilutions for each drug. From the maintained stock cultures of required organisms, 5 µl was taken and added into 2 ml of BHI broth. In each serially diluted tube, 200 µl of the above culture suspension was added. The tubes were kept in a carbon dioxide jar (CO_2) for *Aggregatibacter actinomycetecomitans* and *Actinomycetes* and an anaerobic jar for *Porphyromonas gingivalis* and *Streptococcus mitis* for 72 hours. These tubes were then incubated for 24 hours at 37 ^{0}C. The last tube with clear supernatant was considered without any growth in each series of tubes and taken as MIC value. Turbidity in the MIC tube indicated the growth of the bacteria implying that the bacteria are resistant to mouthwashes. Therefore, the MIC was taken as the lowest concentration that prevented the growth of the bacteria (Figure 1).

Determination of Time Kill Assay

Time-kill curves monitor bacterial growth and death over various antimicrobial concentrations. Thus, it helps to evaluate the effect of antimicrobials over time.

Procedure: Each mouthwash in equal quantity was mixed with a mixture of organisms, including *Streptococcus mitis*, *Aggregatibacter actinomycetecomitans*, *Actinomycetes* and *Porphyromonas gingivalis.* These were then plated immediately, and the time was noted as 0 min. These tubes were kept in a CO_2 jar until the next time slot. At subsequent time points, which were 5 mins, 10 mins and 30 mins, the tubes were removed, and plating was done. These plates were incubated according to the growing requirement, i.e., in a CO_2 and anaerobic jar. After 48-72 hours of incubation, the plates were removed, following which colony count was noted (Figure 2).

Plaque Sample Collection

Gingival Index (Loe and Sillness,1963) and Plaque Index (Sillness and Loe, 1964) were recorded at the baseline. Fifteen (n=15) plaque samples were collected from fifteen subjects diagnosed with moderate to severe gingivitis. The site was air-dried and isolated with cotton rolls. Supragingival plaque samples were collected from buccal and lingual surfaces of both arch using curettes. From each subject, the total accumulated plaque sample was divided into three and was then immediately transferred into three sterile plastic vials containing reduced transport fluid which was sent to the laboratory for processing. The mouthwashes were categorized into three groups: Group I - *Albizia lebbeck* (L.) Benth. mouthwash, Group II - *Bauhinia variegtata* Linn. mouthwash and Group III - Chlorhexidine mouthwash.

Microbiological Testing Procedure

After receiving the plaque sample, the sample was diluted in 1:100 dilution, which consisted of 99 µl of thioglycollate broth and 1 µl of plaque sample. This was mixed and inoculated on blood agar which was considered as pre-mouthwash sample. After this, in a separate Eppendorf tube, 200µl of respective mouthwash was taken, and 100 µl of diluted plaque sample was added. This was kept for half an hour and then inoculated on blood agar. This was considered a post-mouthwash sample. The plates were inoculated in an anaerobic jar, and the colony count was recorded after three days.

Statistical Analysis

Data were entered in Microsoft Excel and analyzed using SPSS for Windows, Version 17 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to calculate standard deviation, percentages, and mean values. Analysis of variance (ANOVA) was applied to know whether the differences in the colony-forming unit (CFU) of the three groups being compared were statistically significant or not. Bonferroni post hoc test was used to know the difference between the mouthwashes. Analysis of covariance (ANCOVA) was also done to adjust for the baseline differences. Student's paired t-test was applied to know whether the differences in the CFU of the three groups before and after the intervention was statistically significant or not. A p-value of less than 0.05 was taken as statistically significant.

RESULTS

The broth dilution procedure was utilized to compare the effects of these herbal mouthwashes and Chlorhexidine on *Aggregatibacter actinomycetecomitans, Streptococcus mitis*, *Porphyromonas gingivalis* and *Actinomycetes* strains (Table 1).

Time Kill Curve

The minimum time required to inhibit the growth of periodontal pathogens in the study was drawn at their MIC (Table 2).

Intergroup Comparison

It was found that *Aggregatibacter actinomycetecomitans* for Group I at its MIC of 6.25 µg/ml showed a gradual decrease in growth but failed to show no growth after 30 minutes when compared to Group II and Group III, which showed sensitivity at 12.5 µg/ml and 0.8 µg/ml respectively. For *Streptococcus mitis*, Group I and Group II at its MIC 12.5 µg/ml and 25 µg/ml, respectively, showed a gradual decrease in their growth, whereas Group III at MIC of 25 µg/ml showed absolutely no growth at 10 minutes. For *Porphyromonas gingivalis,* no growth was seen in all mouthwashes at its MIC 0.8 µg/ml for Group I and 0.4 µg/ml for Group II and Group III at 30 minutes. For *Actinomycetes*, Group I at its MIC 12.5 µg/ml showed no growth at 10 minutes. Group II at 6.25 µg/ml showed a gradual decrease in its growth at 30 minutes, whereas Chlorhexidine at MIC of 0.8 µg/ml showed no growth at 10 minutes.

The mean age among the groups was 24.07±8.82. The assessed parameters include plaque and gingival indexes, whose mean values were 1.41±0.25 and 1.58±020, respectively (Table 3).

The total number of participants was fifteen, consisting of four males and eleven females. The gender distribution among male and females were 26.7% and 73.3%, respectively (Table 4).

Intragroup Comparison

Group I: There was a significant reduction in mean colony forming unit (CFU) values when the before value (440±55.42) was compared with the after value (318.20±50.78). The reduction was statistically significant when compared with paired t-test (p<0.001) (Table 5, Graph 1).

Group II: There was a significant reduction in mean CFU values when the before value (476.13±86.99) was compared with the after value (353.93±68.19). The reduction was statistically significant when compared with paired t-test (p<0.001) (Table 5, Graph 1).

Group III: There was a significant reduction in mean CFU values when the before value (377.47±50.93) was compared with the after value (47.20±46.62). The reduction was statistically significant when compared with paired t-test (p<0.001) (Table 5, Graph 1).

Intergroup Comparison

When the three groups were compared with each other using one-way ANOVA, there was a significant difference between the three groups in mean CFU values at baseline (Table 5). Similarly, significant differences were also observed between the groups for after values. Hence pair-wise comparison by post hoc Bonferroni test was done (Table 6).

ANOVA: Analysis of covariance was done by adjusting for the post-mouthwash sample values by taking baseline values as covariants. Differences between the groups in after-sample values remain statistically significant (f ratio-126.33, p-value <0.001s) even after adjusting the values before the sample. Hence the differences in the baseline values between the groups did not significantly change the conclusions for the post-mouthwash sample values.

Pre-Mouthwash Sample

Group I v/s Group II: Pair-wise comparison between group I and group II gave a mean difference of -36.07, and post hoc comparison showed that the difference was not statistically significant (p-value- 0.43ns) (Table 6).

Group I v/s Group III: Pair-wise comparison between groups I and III showed statistically significant differences between the mean values (p value-0.04) (Table 6). The mean CFU in group I (440±55.42) was significantly higher compared to group III (377.47±50.93) (Table 5).

Group II v/s Group III: Pair-wise comparison between group II and group III showed statistically significant differences between the mean values (p-value <0.001) (Table 6). The mean CFU in group II (476.13±86.99) was significantly higher compared to group III (377.47±50.93) (Table 5).

Post-Mouthwash Sample

Group I v/s Group III: Pair-wise comparison between group I and group III showed a statistically significant between the mean values (p-value<0.001) (Table 6). The mean CFU in group III (47.20±46.62) was significantly lower when compared to group I (318.20±50.78) (Table 5).

Group I v/s Group II: Pair-wise comparison between group I and group II gave a mean difference of -15.09, and post hoc comparison showed that the difference was not statistically significant (p-value >0.05ns) (Table 6).

Group II v/s Group III: Pair-wise comparison between group II and group III showed statistically significant differences between the mean values (<0.001) (Table 6). The mean CFU in group II (353.93±68.19) was significantly higher compared to group III (47.20±46.62) (Table 5).

Table 1: Effects of *Albizia lebbeck* (L.) Benth. mouthwash, *Bauhinia variegata* Linn. mouthwash and Chlorhexidine on a set of standard laboratory strains using Broth Dilution method

Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
Aggregatibacter actinomycetecomitans										
Albizia lebbeck	S	S	S	S	S	R	R	R	R	R
Bauhinia variegata	S	S	S	S	R	R	R	R	R	R
Chlorhexidine	S	S	S	S	S	S	S	S	R	R
Streptococcus mitis										
Albizia lebbeck	S	S	S	S	R	R	R	R	R	R
Bauhinia variegata	S	S	S	R	R	R	R	R	R	R
Chlorhexidine	S	S	S	R	R	R	R	R	R	R
Porphyromonas gingivalis										
Albizia lebbeck	S	S	S	S	S	S	S	S	R	R
Bauhinia variegata	S	S	S	S	S	S	S	S	S	R
Chlorhexidine	S	S	S	S	S	S	S	S	S	R
Actinomycetes										
Albizia lebbeck	S	S	S	S	R	R	R	R	R	R
Bauhinia variegata	S	S	S	S	S	R	R	R	R	R
Chlorhexidine	S	S	S	S	S	S	S	S	R	R

S- Sensitive, R-Resistant

Table 2: Minimum time required to inhibit the growth of Periodontal pathogens in the study (time-kill curve)

Samples	00 min	05 min	10 min	30 min
Albizia lebbeck				
Aggregatibacter actinomycetecomitans	30	19	16	14
Streptococcus mitis	>300	238	193	136
Bauhinia variegata				
Aggregatibacter actinomycetecomitans	>350	270	08	nil
Streptococcus mitis	49	36	06	05
Actinomycetes	62	41	10	04
Chlorhexidine				
Aggregatibacter actinomycetecomitans	35	18	NG	NG
Streptococcus mitis	110	84	NG	NG
Actinomycetes	54	20	NG	NG

NG - No growth

Table 3: Descriptive statistics of Age, Plaque Index and Gingival Index among study groups

Variables	Mean	Standard deviation
Age (years)	24.07	8.82
Plaque index	1.41	0.25
Gingival index	1.58	0.20

Table 4: Gender distribution of study participants

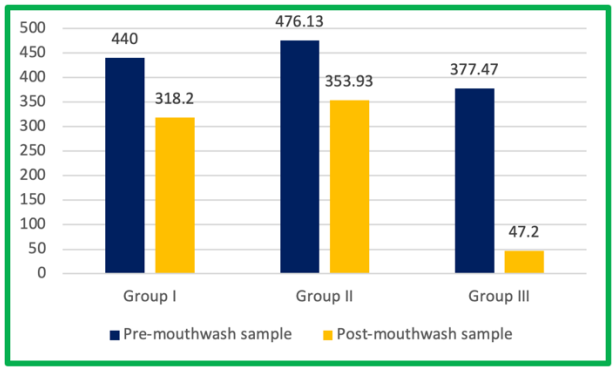
Gender	Number	Percentage
Male	4	26.7%
Female	11	73.3%
Total	15	100%

Table 5: Comparison of reduction in colony forming units at baseline (Pre-mouthwash sample) and after 30 minutes (Post-mouthwash sample) in each group

	Group I	Group II	Group III
Pre-mouthwash sample	440±55.42	476.13±86.99	377.47±50.93
Post-mouthwash sample	318.20±50.78	353.93±68.19	47.20±46.62
Mean difference	121.87±37.33	122.20±44.05	330.27±64.47
t-value	12.64	10.74	19.84
p-value	<0.001	<0.001	<0.001

Table 6: Multiple comparisons by analysis of variance followed by Post Hoc Bonferroni test

Pair-wise comparison by Post Hoc Bonferroni test				Overall Comparison ANOVA	
Groups	**Groups**	**Mean difference**	**p-value**	**f- ratio**	**p-value**
Group I (Pre-mouthwash sample)	Group II	-36.07	0.43ns	8.47	0.001, s
Group I (Post-mouthwash sample)	Group II	-15.09	>0.05ns	134.65	<0.001
Group I (Pre-mouthwash sample)	Group III	62.60	0.04s	8.47	0.001, s
Group I (Post-mouthwash sample)	Group III	235.18	<0.001s	134.65	<0.001
Group II (Pre-mouthwash sample)	Group III	98.66	0.001s	8.47	0.001, s
Group II (Post-mouthwash sample)	Group III	250.27	<0.001s	134.65	<0.001



Graph 1: Comparison of CFU at baseline and after 30 minutes in each group

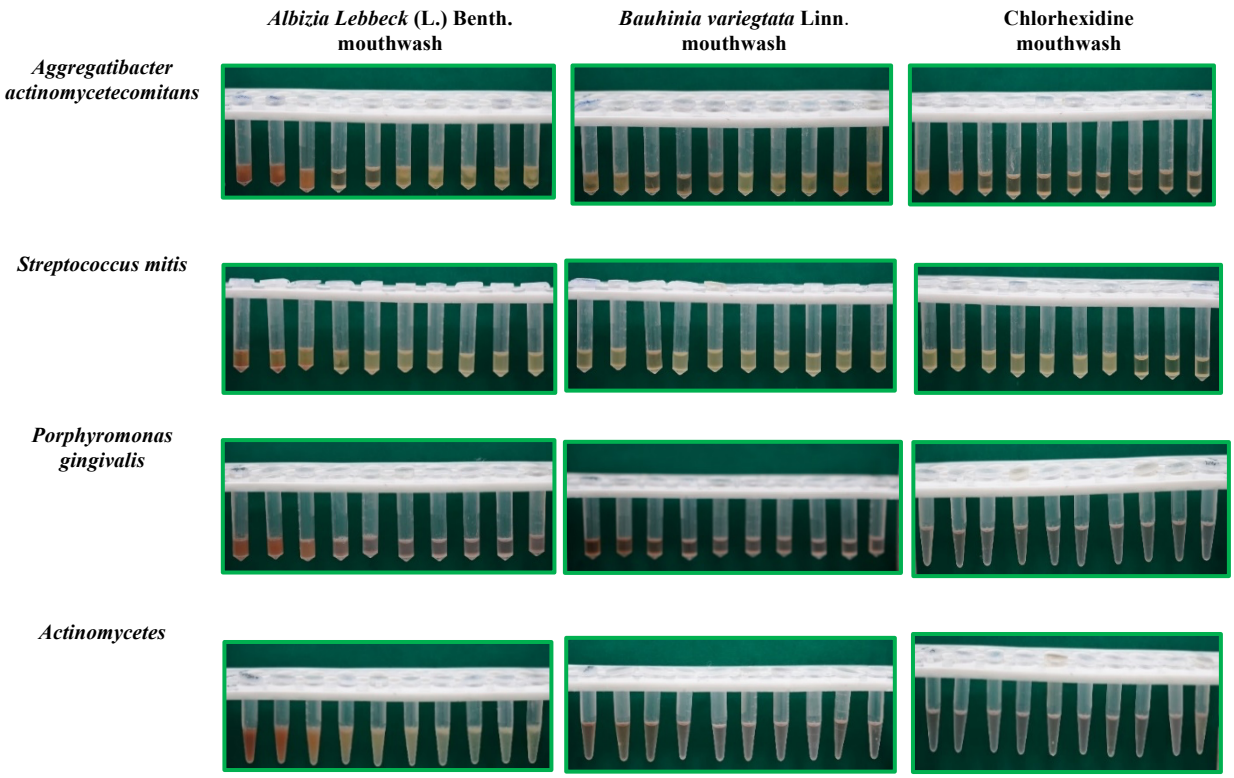


Figure 1: MIC of *Albizia lebbeck* (L.) Benth. Mouthwash, *Bauhinia variegata* Linn. Mouthwash and Chlorhexidine Mouthwash

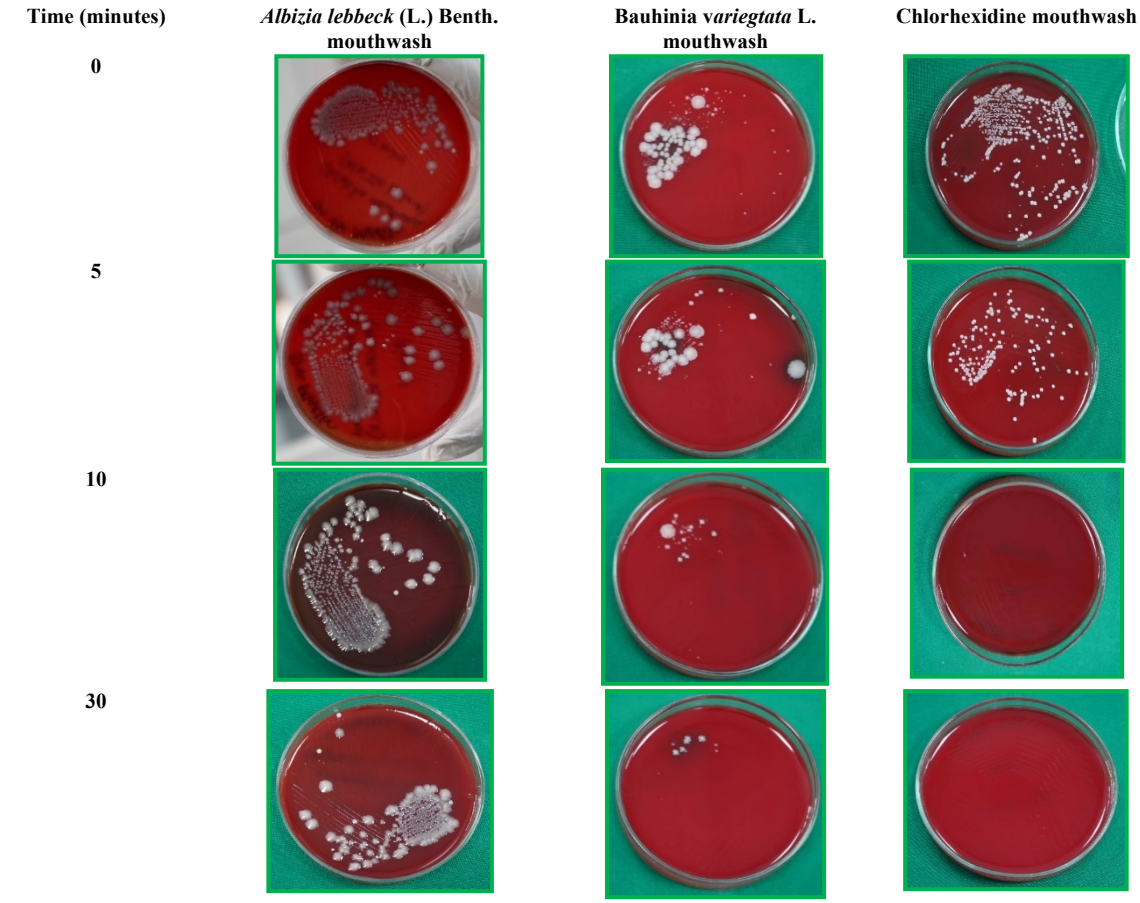


Figure 2: Time Kill Assay of *Albizia lebbeck* (L.) Benth. Mouthwash, *Bauhinia variegata* Linn. Mouthwash and Chlorhexidine Mouthwash

DISCUSSION

The current study comprised *in-vitro* tests for determining the MIC of *Albizia lebbeck* (L.) Benth., *Bauhinia variegata* Linn. and Chlorhexidine mouthwashes. Then the antibacterial action of these mouthwashes on the supragingival plaque from moderate to severe gingivitis subjects was determined and compared.

Herbal medicines from time immemorial have been known to have therapeutic benefits on various plant constituents.[7] World Health Organization computed that around 70–80% of the society favours herbal therapeutic agents for treating various ailments.[8] This is assumed owing to its biological activity, advanced safety margin, increased antimicrobial resistance and low cost. The biological activity of the herbs, such as antibacterial, anti-inflammatory, and antioxidant properties are due to the presence of biologically active compounds such as flavonoids, coumarins, glycosides, phenolic acids, resins, phytoesters, choline, carotenoids, tannins, vitamins, mineral salts such as magnesium, iron, lithium and essential oils.[9] These properties thus benefit gingival health compared to synthetic chemicals' antimicrobial mechanisms.

In the present study, *Albizia lebbeck* (L.) Benth., *Bauhinia variegata* Linn. and Chlorhexidine (CHX) mouthwashes were tested against the following bacterial strains, which include *Streptococcus mitis*, *Aggregatibacter actinomycetecomitans*, *Actinomycetes* and *Porphyromonas gingivalis* that contribute to gingivitis. The MIC by broth dilution technique was assessed. The MIC value for Group I, II and III mouthwash ranged 0.8 µg/ml-12.5 µg/ml, 0.4 µg/ml-25 µg/ml and 0.4 µg/ml-25 µg/ml, respectively.

Time kill test was used in this study to determine the antimicrobial effects of these mouthwashes over time. It was observed that herbal mouthwashes showed a gradual decrease in the growth of the strains compared to Chlorhexidine.

In the study, the antibacterial efficacy of these mouthwashes on the supragingival plaque was also assessed. The study comprised 15 participants, four males and eleven females. The distribution of the participants according to age was between 17 and 52 years, with the mean being 24.07. The mean plaque index and gingival index scores were 1.41 and 1.58, respectively. The existence of plaque is known to be the cause of gingivitis.[10] Gingival index was chosen to select participants with moderate to severe gingivitis.

It was also evident in the study that there was a significant reduction in CFU values of Group I, Group II and Group III mouthwashes compared to the pre-mouthwash sample values of the plaque sample. The CFU value of Group I at baseline was 440±55.42. After 30 minutes, the CFU values were noted to be 318.20±50.78. This indicates the possible antibacterial action of *Albizia lebbeck* (L.) Benth., mouthwash. The main constituents of the *Albizia lebbeck* (L.) Benth. bark are condensed tannins, d-catechin, lebbecacidin, anthraquinone glycoside, isomers of leucocyanidin, and friedelin-3-one. The anthraquinone glycosides from the bark are active against aerobes. This glycoside content causes leakage of cytoplasmic constituents. Thus, it is attributed to antimicrobial activity.[11] It has been reported that the MIC values of the methanol extract of *Albizia lebbeck* (L.) Benth., against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* to be 0.01 mg/ml, indicative of the extract's antimicrobial activity.[12] These findings were supported by a study by Chulet R *et al.* 2010, wherein ethyl acetate successive extract showed antibacterial activity against gram-positive and gram-negative bacteria *in-vitro*.[4] In another study, the methanolic extracts of *Albizia lebbeck* (L.) Benth. illustrated inhibitory activity against the pathogens, namely *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus,* whereas the ethyl acetate extract demonstrated inhibition against *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia*.[13] It was noted in our study that the inhibitory effect of the herb was less when compared to Chlorhexidine.

The CFU value of Group II at baseline was 476.13±86.99. After 30 minutes, the CFU was noted to be 353.93±68.19. This signifies the possible antibacterial action of *Bauhinia variegata* Linn. mouthwash. History reports this herb as a drug with good medicinal value.[14] These plants possess various curative properties due to secondary metabolites. *Bauhinia variegata* Linn. consist of anthraquinones derivatives, cardenolides and cardiac glycosides, flavonoids, resins, saponins and tannins that are known to have various curative effect against pathogenic organisms. Kumar *et al.* 2005, have reported that this herb's broad spectrum of antimicrobial activity is due to the presence of phenol metabolites.[5] This finding is supported by Pandey S *et al*. 2015, who reported that methanolic hydro extract of *Bauhinia variegata* Linn. inhibited microbial growth dose-dependently.[15] Another study by Patil *et al.* 2015, reported the antibacterial activity of this herb against *Staphylococcus aureus* and *Escherichia coli*.[16] Nabu Raj *et al.* reported that ethanolic extract of the stem bark of *Bauhinia variegata* Linn. was found to have antimicrobial activity against *Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus* and *Vibrio cholerae*.[17] The therapeutic value of plants lies in the bioactive phytocomponents present in the plants.[18] However, when the antibacterial effect was compared with Chlorhexidine, the inhibitory effect of this herb was less.

The CFU values of Group III at baseline were 377.47±50.93. After 30 minutes, the CFU was noted to be 47.20±46.62. This signifies the antibacterial action of Chlorhexidine mouthwash. This bactericidal effect is due to the cationic structure penetrating the cell membrane leading to cytoplasmic coagulation.[19] As evident from the study results, there were statistically significant differences between Chlorhexidine and herbal mouthwashes. However, Chlorhexidine is accompanied by some side effects like a bitter taste, formation of extrinsic stains on the teeth and tongue, increased risk of caries due to fermentation and alcohol content, altered taste perception, metallic taste, cytotoxic effects on cells, unilateral or bilateral parotid swelling and enhanced supragingival calculus formation.[20] All these disadvantages have led current research to more natural and biocompatible agents.

The present study showed marked antibacterial activity by Group I and Group II but is not as significant as Group III. This could be attributed to a broad-spectrum antiseptic with a pronounced antimicrobial effect of Chlorhexidine. Furthermore, the present study detected no statistical difference between Group I and Group II, suggesting similar functional activity. The reduction in CFU can be attributed to the presence of bioactive ingredients in the herbs.

To our knowledge, this is the first study of its kind to check the antibacterial activity of these herbs against periodontopathogens.

CONCLUSION

The MIC of *Albizia lebbeck* (L.) Benth., *Bauhinia variegata* Linn. and Chlorhexidine mouthwashes ranged 0.8 µg/ml-12.5 µg/ml, 0.4 µg/ml-25 µg/ml and 0.4 µg/ml-25 µg/ml against following

bacterial strains which include *Streptococcus mitis*, *Aggregatibacter actinomycetecomitans*, *Actinomycetes* and *Porphyromonas gingivalis.*

It was evident in the study that *Albizia lebbeck* (L.) Benth., *Bauhinia variegata* Linn. and Chlorhexidine mouthwashes exhibited antibacterial activity. When the antibacterial property of *Albizia lebbeck* (L.) Benth. and *Bauhinia variegata* Linn. mouthwash was compared with Chlorhexidine; the inhibitory effect of the herbs was less efficacious. Furthermore, the present study detected no statistical difference between the antibacterial property of *Albizia lebbeck* (L.) Benth. and *Bauhinia variegata* Linn. mouthwashes. Thus, it can be concluded from the study that herbal mouthwashes were less potent when compared to Chlorhexidine.

Further research could study the antimicrobial efficacy of herbal mouth rinse in greater depth, and *in vivo,* clinical testing is essential to confirm the *in-vitro* results.

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