RESEARCH ARTICLE

Comparison of the Antibacterial Efficiency of Herbal Extracts of Aloe Vera Leaves and Mushroom against *Streptococcus mutans* and *Lactobacillus*: An *In Vitro* Study

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Abstract

Background and objectives: This study evaluated and compared the antimicrobial efficacy and minimal inhibitory concentration (MIC) of chlorhexidine (CHX) 0.12% and extracts of aloe vera and mushroom against *Streptococcus mutans* and *Lactobacillus*.

Materials and methods: The agar disk diffusion and the broth microdilution method were used to check the antimicrobial activity of 0.12% CHX and aqueous extracts of two medicinal plants. The test samples were divided as follow: *S. mutans*, group I: CHX, group II: aloe vera, and group III: mushroom. *Lactobacillus*, group I: CHX, group II: aloe vera, and group III: mushroom. The zone of inhibition and MIC values were tabulated and the data were statistically analyzed using ANOVA and Bonferroni *post hoc* tests.

Results: Chlorhexidine shows maximum antibacterial action against *S. mutans* and *Lactobacillus* followed by the mushroom extract and then aloe vera extracts with the zones of inhibition of (20.3 mm/24.13), (17.03/16.05), and (14.09/14.26), respectively. Both the extracts had MIC value of 80 µg/mL and CHX was 40 µg/mL.

Conclusion and inference: Within the limitations of this *in vitro* study, it can be concluded that all the herbal extracts tested in this study demonstrated antibacterial activity against mutans streptococci (MS) and *Lactobacillus*. Chlorhexidine shows maximum antibacterial action against *S. mutans* and *Lactobacillus* followed by the mushroom extract and then aloe vera extracts with the zones of inhibition of (20.3 mm/24.13), (17.03/16.05), and (14.09/14.26), respectively. Herbal products have potent antimicrobial activity that can be looked at as an alternative to CHX. However, further *in vitro* and long-term *in vivo* studies are recommended to confirm and correlate the findings of this study to a clinical situation.

Keywords: Chlorhexidine, *Lactobacillus*, *Streptococcus mutans*.

International Journal of Experimental Dental Science (2020): 10.5005/jp-journals-10029-1203

Introduction

Dental caries is one of the common infectious diseases of the oral cavity. Mutans streptococci (MS) and *Lactobacillus* are the main microorganisms in the etiology of dental caries. Many attempts have been made to eliminate them from the oral cavity. Antimicrobial mouthwashes are one of the commonly used methods to inhibit plaque formation, reduce gingival inflammation, and prevent dental caries. Herbs extracts contain wide variety of active components, which have antimicrobial, anti-inflammatory, antioxidant, and biocompatible properties. Hence in search of a novel antimicrobial agent, this study was done to evaluate and compare the antimicrobial efficacy and minimal inhibitory concentration (MIC) of chlorhexidine (CHX) 0.12% and extracts of two different herbs, mushroom, aloe vera, on *Streptococcus mutans* and *Lactobacillus*.

Dental caries is one of the common infectious diseases of the oral cavity.[1] It is an irreversible process that causes demineralization of inorganic and destruction of the organic substance of the tooth leading to cavitations. It has a multifactorial etiology that depends on the presence of bacterial plaque, sugars, and quantity and quality of saliva.[2]

The literature suggests that mutans streptococci, gram-positive facultative anaerobes, are the major pathogen and the initiator of dental caries (induce caries formation in animals fed with a sucrose-rich diet).[3] Mutans streptococci have been isolated from cavitated carious lesions. They are highly acidogenic and aciduric, able to produce surface antigens I/II and water-insoluble glucan, which promote bacterial adhesion to both the tooth surface and other bacteria.[4] Lactobacilli, gram-positive, nonspore-forming rods that grow best under microaerophilic conditions, are also acidogenic, aciduric, and multiply at low pH. They are found in pit and fissure and deep dentinal caries, which favor their retention and thus help in progression of caries.[2]

Mutans streptococci metabolize dietary sugar to produce a sticky, extracellular, dextran-based polysaccharide, thus forming dental plaque. This is a biological film where various bacterial species interact and form a protective barrier against antimicrobial agents used in clinical dentistry.[4] Controlling the levels of these microorganisms in the dental plaque will aid in the prevention of caries. Hence, a caries prevention program primarily should be aimed at reducing the cariogenic bacterial plaque.[5]

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How to cite this article: Devarasanahalli SV, Kurian B, Aswathanarayana RM, *et al.* Comparison of the Antibacterial Efficiency of Herbal Extracts of Aloe Vera Leaves and Mushroom against *Streptococcus mutans* and *Lactobacillus*: An *In Vitro* Study. Int J Experiment Dent Sci 2020; 9(1):8–12.

Source of support: Nil

Conflict of interest: None

Many attempts have been made to eliminate MS and *Lactobacillus* from the oral cavity. Antimicrobial mouthwashes are one of the commonly used methods to inhibit plaque formation, reduce gingival inflammation, and prevent dental caries.

Chlorhexidine is the most commonly used mouth rinse and is a gold standard against which other antimicrobial agents are compared.[6] Chlorhexidine is a bisbiguanide that has bactericidal activity against both gram-positive and gram-negative bacteria. It is positively charged and hence binds to various surfaces including enamel pellicle, hydroxyapatite, and mucus membranes that are negatively charged. It has been studied extensively and is currently the most potent chemotherapeutic agent against MS and *Lactobacillus*.[7]

However, its excessive use can result in alterations of the oral flora and development of bacterial tolerance along with certain undesirable side effects like unpleasant taste, staining of teeth and tongue, and restoration, desquamation, and soreness of the oral mucosa.[8] These problems necessitate further research for alternate antimicrobial agents that are safe and potent against specific oral pathogens.

Herb extracts contain wide variety of active components, which have antimicrobial, anti-inflammatory, antioxidant, and biocompatible properties. The advantage of herbal medicine is that it is less likely to cause allergies and side effects.[9]

Mushroom extracts have a wide range of biomolecules with nutritional and medicinal substances with anti-inflammatory and antimicrobial properties. Studies have shown that the mushroom extracts have antimicrobial activity against gram-positive bacteria.[10]

Aloe barbadensis Miller has a long history of use as a therapeutic agent with many reported medicinal properties. Among its therapeutic properties, it has been shown to have anti-inflammatory, immune-stimulatory, cell growth-stimulatory, antibacterial, antifungal, and antiviral properties.[11]

Hence in search of a novel antimicrobial agent, this study was done to evaluate and compare the antimicrobial efficacy and minimal inhibitory concentration (MIC) of CHX 0.12% and extracts of two different herbs, mushroom and aloe vera, on *S. mutans* and *Lactobacillus* (Tables 1 and 2).

Materials and Methods

About 50 g of dried herbs was boiled in 500 mL of deionized distilled water to a final volume of 10–20 mL. The concentrated mixture was filtered and stored in a refrigerator for antimicrobial studies.

Pure strains of MS (ATCC 25175) and *Lactobacillus* (ATCC 11975) were obtained from a standard microbiology lab and maintained at 4°C on slopes of the nutrient agar medium. Active cultures were freshly prepared before every antibacterial assay. A 0.5 McFarland standard was used for visual comparison to adjust the bacterial suspension to a density approximately equivalent to 1.5×10^8 CFU/mL. An antimicrobial assay was performed against the pathogenic strain by the agar disk diffusion method. The groups were divided as follows:

Streptococcus mutans groups:
Group I: Positive control—0.12% CHX solution
Group II: Aqueous extract of aloe vera
Group III: Aqueous extract of mushroom

Lactobacillus groups:
Group I: Positive control—0.12% CHX solution
Group II: Aqueous extract of aloe vera
Group III: Aqueous extract of mushroom

0.1 mL (0.5 McFarland standards) of microbial suspensions was spread evenly onto the surface of the sterilized Mueller Hinton Agar (MHA) agar plates using a sterile glass spreader. The plates were dried for 3–5 minute. Sterile filter paper disks of 6 mm diameter were saturated with 50 µL of each extract. These were air-dried to remove any residual solvent. Three MHA plates inoculated with test bacteria were used for every group. Four disks of each extract were then placed on the surface of each MHA plate ($n = 12$) and incubated at 37°C. After 24 hours of incubation, each plate was examined for inhibition zones that were measured with the Vernier caliper. The bigger the diameter of the inhibition zone, the more susceptible is the microorganism to the antimicrobial agent. Chlorhexidine was used as a positive control against MS and *Lactobacillus*. The minimal inhibitory concentration was determined for two plant extracts against *Streptococcus mutans* and *Lactobacillus* using the ELISA microdilution method.

Results

The statistical analysis was done using the analysis of variance (ANOVA) technique and multiple comparisons were done using the Bonferroni test (*post hoc* test).

Higher mean zone of inhibition for *S. mutans* was recorded in group I followed by group III and group II, respectively. The difference in mean *S. mutans* among the groups was found to be statistically significant ($p < 0.001$) (Fig. 1).

Table 1: Comparison of mean *S. mutans* among the groups

Streptococcus mutans	*Mean*	*Std. dev.*	*SE of mean*	*95% CI for mean*		*Min*	*Max*
				Lower bound	*Upper bound*		
Grou p I	20.31	1.66	0.48	19.25	21.36	18.00	23.20
Group II	14.09	0.95	0.27	13.49	14.70	12.40	16.00
Group III	17.03	0.69	0.20	16.59	17.46	15.80	18.20

Source of variation	*df*	*Sum of squares*	*Mean square*	*F*	*p value*
Between groups	2	232.127	116.063	84.102	<0.001*
Within groups	33	45.541	1.380	–	–
Total	35	277.668	–	–	–

Higher mean zone of inhibition for *S. mutans* was recorded in group I followed by group III and group II, respectively. The difference in mean *S. mutans* among the groups was found to be statistically significant ($p < 0.001$). Multiple comparisons results using the Bonferroni method are given
*Denotes significant difference

Table 2: Comparison of mean *Lactobacillus* among the groups

				95% CI for mean			
Lactobacillus	*Mean*	*Std dev.*	*SE of mean*	*Lower bound*	*Upper bound*	*Min.*	*Max.*
Group I	24.13	1.00	0.29	23.49	24.76	22.70	26.50
Group II	14.26	0.82	0.24	13.74	14.78	12.80	15.60
Group III	16.05	0.70	0.20	15.60	16.50	14.80	17.20

Source of variation	*df*	*Sum of squares*	*Mean square*	*F*	*p value*
Between groups	2	663.067	331.534	458.886	<0.001*
Within groups	33	23.842	0.722	–	–
Total	35	686.909	–	---	–

Higher mean *Lactobacillus* was recorded in group I followed by group III and group II respectively. The difference in mean *Streptococcus mutans* among the groups was found to be statistically significant ($p < 0.001$).
Multiple comparisons results using Bonferroni method are given.
*Denotes significant difference

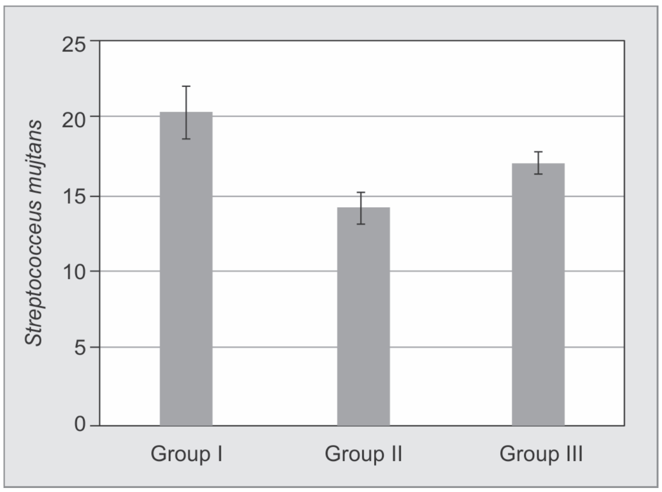

Fig. 1: The difference in mean *S. mutans* was found to be statistically significant between group I and group II ($p < 0.001$), group I and group III ($p < 0.001$), as well as between group II and group III ($p < 0.001$)

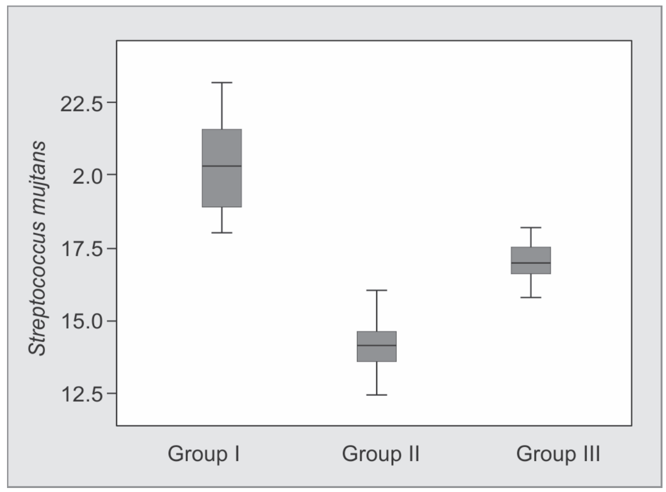

Fig. 2: The box plot representation of *S. mutans* mean difference among three groups

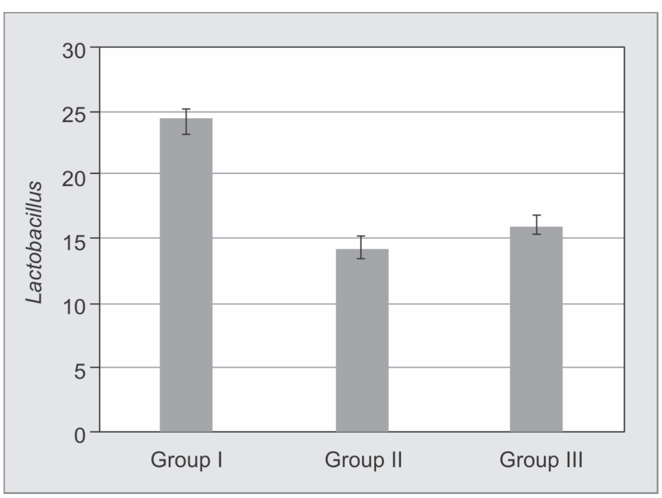

Fig. 3: The difference in mean *Lactobacillus* was found to be statistically significant between group I and group II ($p < 0.001$), group I and group III ($p < 0.001$) as well as between group II and group III ($p < 0.001$)

The difference in mean *S. mutans* was found to be statistically significant between group I and group II ($p < 0.001$), group I and group III ($p < 0.001$), as well as between group II and group III ($p < 0.001$) (Figs 1 and 2).

Higher mean *Lactobacillus* was recorded in group I followed by group III and group II, respectively. The difference in mean *S. mutans* among the groups was found to be statistically significant ($p < 0.001$) (Fig. 3).

The difference in mean *Lactobacillus* was found to be statistically significant between group I and group II ($p < 0.001$), group I and group III ($p < 0.001$), as well as between group II and group III ($p < 0.001$) (Figs 3 and 4).

Discussion

Dental plaque is a biofilm, i.e., a group of microorganisms embedded in a polysaccharide matrix attached to the tooth surface.[4] Epidemiological studies have shown that tooth decay is the most common squeal of dental plaque formation.[5]

Mutans streptococci were chosen as the test organism because they are one of the predominant inhabitants of dental plaque and have been implicated in the formation of dental caries because

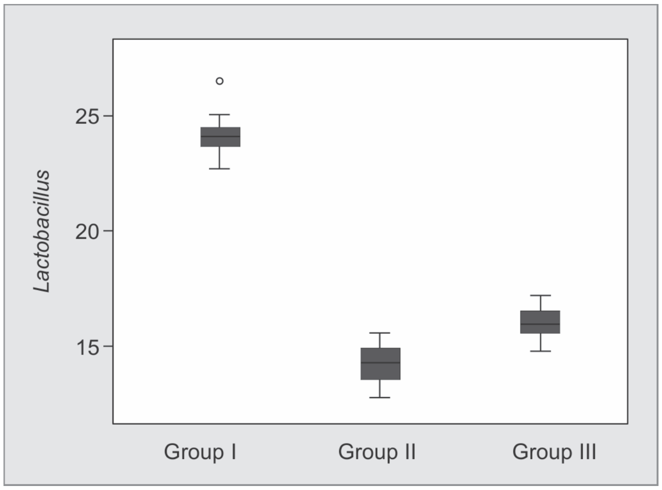

Fig. 4: The box plot representation of *Lactobacillus* mean difference among three groups

of their acidogenic and aciduric properties. Mutans streptococci adhere by hydrophobic bonds to the enamel surface and ferment dietary carbohydrates, notably sucrose. Acid production resulting from carbohydrate metabolism by these bacteria subsequently decreases the environmental pH and is responsible for the demineralization of tooth surfaces.[12] Mutans streptococci play a critical role in destabilizing the homeostasis of the plaque by facilitating a shift of the demineralization/remineralization balance from "net mineral gain" to "net mineral loss" (acidogenic stage). Once the acidic environment has been established, MS and other aciduric bacteria may increase and accumulation of acids in the dental plaque initiates dissolution of the tooth enamel subsequently leading to localized decalcification, cavitations, and breakdown of calcified dental by sustaining an environment characterized by "net mineral loss" (aciduric stage).[13]

Lactobacillus acidophilus has been isolated from carious teeth and its number in plaque has been shown to increase only after caries has developed. Lactobacilli are relevant secondary pathogen involved in the progression of caries.[14]

The removal of bacterial biofilms is a decisive component in the prevention and treatment of dental caries. The levels of microorganisms in the biofilm should be reduced for controlling plaque. Thus, several methods to reduce the amount of dental plaque include mechanical and chemical methods. The use of mechanical agents is a simple cost-effective method. The effectiveness of this method however is influenced by the individual's dexterity and motivation. Mouthwashes are the commonly advocated chemical method for plaque elimination. Mouth rinsing is favored because of its ease of use and instant breath-freshening effect. Among all available mouthwashes, CHX has good antimicrobial property.[15] Prolonged use of these has certain adverse effects such as alterations of normal flora, unpleasant taste, staining of teeth and restorations, soreness in the oral mucosa, etc. So, researchers are trying to pay more attention to herbal drugs that are a good alternative to synthetic chemical substances for caries prevention. Herbs have shown to possess antibacterial, antiviral, insecticidal, and antioxidant properties due to the presence of wide variety of active phytochemicals, including flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides. Their antimicrobial action is basically on the cell membrane, which disrupts its structure, blocks membrane synthesis, and inhibits cellular respiration, thereby causing cell leakage and death.[16]

In the current study, we tested two medicinal plants aloe vera and mushroom for their antimicrobial effectiveness against MS and lactobacilli. For antibacterial susceptibility testing, disk diffusion and broth microdilution methods were followed as per the NCCLS guidelines.[17]

Disk diffusion is a qualitative test. Diameter of the zone of growth inhibition around an antibiotic disk predicts the effectiveness of the antimicrobial agent. Broth microdilution is a quantitative method used for determining the MICs of antimicrobial agents and is considered to be "gold standard" reference testing methodology against which other methods, such as disk diffusion, are calibrated.

In present study, CHX showed better zones of inhibition than other groups. Chlorhexidine gluconate, which is charged positively, shows high affinity for negative ions found in cell membranes of microorganisms. It indirectly affects the enzymatic function of dehydrogenase and adenosine triphosphatase present in the cell wall of bacteria resulting in the disruption of the cell membrane. It is evident in this study that CHX showed a definite reduction in the microbial activity, which has marked anticarcinogenic effect. However, the side effect of CHX may limit the long-term use of CHX.[18,19]

In this study, the aqueous extract of aloe vera and mushroom was chosen, based on the results of the pilot study, which was conducted using different solvents like methanol, water, and chloroform.

Higher mean *S. mutans* and *Lactobacillus* was recorded in group I followed by group III and group II, respectively. The difference in mean *S. mutans* among the groups was found to be statistically significant ($p < 0.001$). Multiple comparisons results using the Bonferroni method are given below:

The mushroom extract showed higher antibacterial activity against MS and *Lactobacillus*. Mushroom extracts have some active, low-molecular-weight compounds (plectasin, confuentin, grifolin, neogrfolin) that show antibacterial action.[20–22] A study done by Signoretto et al. showed that low-molecular-weight mass fraction of an aqueous mushroom extract had antimicrobial activity against potential oral pathogens.[23] The literature shows that mushroom has highest activity against gram-positive bacteria and the gel form of extract increases the contact time, which enhances the performance.[20]

Aloe vera contains active ingredients like anthraquinones, tannins, myristic acid, curcumin, and nimbidin that contribute to its antimicrobial action.[24,25] In this study, aloe vera showed less antibacterial activity than mushroom. Molecular weight (1000 kDa) of the aloe vera is more than that of mushroom (12 kDa), which can result in less penetration of aloe vera through the agar medium and hence less action.[26,27]

On reviewing the literature of various studies done previously and on analyzing the results of the present study, it can be inferred that herbal extracts can be a promising substitute for the artificial antimicrobial agents, thereby limiting their side effects. For any mouthwash to be used clinically, there is a need to investigate its substantivity, action on bacterial tolerance, and discoloration on long-term usage. The results of laboratory studies may not reflect the real effect of a material when applied in an *in vivo* condition but *in vitro* research gives support to clinical trials. Further long-term *in vivo* studies need to be conducted before it can be recommended for routine clinical usage.

Conclusion

Within the limitations of this *in vitro* study, it can be concluded:

- All the herbal extracts tested in this study demonstrated antibacterial activity against MS and *Lactobacillus*.
- Chlorhexidine showed maximum antibacterial action against *S. mutans* and *Lactobacillus* followed by mushroom extract and then aloe vera extract with the zones of inhibition of (20.3mm/24.13), (17.03/16.05), and (14.09/14.26), respectively.
- Tested extracts demonstrated antimicrobial activity more against *Lactobacillus* than *S. mutans*.

Herbal products have potent antimicrobial activity that can be looked at as an alternative to chlorhexidine. However, further *in vitro* and long-term *in vivo* studies are recommended to confirm and correlate the findings of this study to a clinical situation.

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