

# A randomized clinical study for comparative evaluation of Aloe Vera and 0.2% chlorhexidine gluconate mouthwash efficacy on de-novo plaque formation

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## Abstract

**Objective:** To comparatively assess the antiplaque efficacy of Aloe vera mouthwash and 0.2% chlorhexidine gluconate mouthwash on de novo plaque formation. **Materials and Methods:** This was a randomized, single blind, parallel, controlled clinical study with 90 healthy participants, with mean age of  $27.19 \pm 12.08$  years. After thorough oral prophylaxis, participants were instructed to discontinue mechanical plaque control. Participants were divided randomly into three groups; pure Aloe vera mouthwash was dispensed to the test group; control group received 0.2% chlorhexidine gluconate mouthwash; in Placebo group, flavored distilled water was used as oral rinse twice daily. Effect on 4-day de novo plaque formation was assessed by comparing pre-rinsing Quigley Hein Modified Plaque Scores were analyzed statistically using analysis of variance and Student's *t*-test. **Results:** Post-rinsing control group showed the least plaque score which was comparable to the test group. Both the control group and test group showed significant difference with the placebo group. **Conclusions:** Herbal mouthwash containing Aloe vera mouthwash has comparable antiplaque efficacy as the gold standard 0.2% chlorhexidine gluconate with fewer side effects and can be considered as an alternative.

**Key words:** *Aloe vera, antiplaque efficacy, chlorhexidine, plaque index*

## INTRODUCTION

Periodontal health is a vital and integral component of overall dental health. Epidemiological studies have demonstrated that, when periodontal disease is uncontrolled, it can act as a crucial factor in tooth loss. The mainstay of prevention of periodontal disease is regular and adequate plaque control using personal oral

hygiene protocol and periodic professional review and maintenance wherever indicated.<sup>[1]</sup>

Though mechanical plaque control is the most effective oral hygiene measure, acceptable plaque removal requires time, motivation, and manual dexterity.<sup>[2]</sup> This in turn has led to a quest for

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chemotherapeutic agents that would inhibit plaque formation. A variety of products with antiplaque properties are a topic of current research. The mode of delivery for these chemical agents is diverse and includes toothpastes, chewing gum, irrigators, spray, varnishes, and mouthwashes.<sup>[3]</sup> Mouthwashes have proved to be a simple, safe, and effective delivery system, and in popularity are next only to toothpastes; mouthwashes have proven to play a vital role in plaque reduction.<sup>[4]</sup>

Chemical agents such as chlorhexidine and triclosan are regularly used as mouth rinses and the later has also been used as an additive in dentifrices for the prevention of plaque formation and thereby gingivitis.<sup>[5]</sup> Chlorhexidine is a gold standard in preventing dental plaque formation. This broad-spectrum antibiotic, a cationic bis-biguanide is extensively used as an antiplaque agent.

Aloe vera, a cactus-like plant, belongs to the Lilaceae family. The core mucilaginous tissue of the Aloe vera leaf is used as a gel for treatment of multiple conditions such as sunburn, wounds, digestive tract disorders, and as a laxative. Pharmacological actions attributable to Aloe vera include antibacterial, antiviral, antifungal, antioxidant, and anti-inflammatory.<sup>[6]</sup>

There is no published data available, to the best of authors' knowledge, that assesses antiplaque efficacy of Aloe vera where the study cohort has been recruited from the general population. Hence, this present study was conducted to compare and evaluate efficacy of Aloe vera mouthwash on clinical levels of dental plaque with established antiplaque agent of 0.2% chlorhexidine gluconate and placebo using the approach of 4-day plaque regrowth.

## MATERIALS AND METHODS

This study was designed as a single blind, randomized, parallel, controlled clinical trial to comparatively evaluate the antiplaque efficacy of placebo, Aloe vera mouthwash, and 0.2% chlorhexidine gluconate mouthwash. The study recruited 90 participants (45 females and 45 males, with age ranging from 18 to 40 years, and mean age of  $27.19 \pm 12.08$  years). The ethical committee of the institution gave ethical clearance for the study, and the study was carried out according to the ethical principles outlined by the World Medical Association's Declaration of Helsinki (revised in 2002).<sup>[7]</sup> The signed informed consent was obtained from all the participants.

### Inclusion criteria

Systemically healthy participants with (1) 20 natural teeth with 5 teeth per quadrant; (2) deficient supragingival plaque and calculus retentive areas.

### Exclusion criteria

Participants with (1) probing pocket depth  $\geq 3$  mm, (2) antimicrobial therapy 1 month prior to the study; (3) using dentifrices or mouth rinses with anti-inflammatory properties; (4) smokers and tobacco consumers; (5) pregnant women; (6) with orthodontic appliances; (7) severely misaligned teeth; (8) fully crowned teeth and removable partial dentures; (9) pharmacological and medical history that could undermine the outcomes of the study.

### Study design

In all the selected participants, oral hygiene status assessment was carried out by plaque index (PI) Quigley and Hein (1962),<sup>[8]</sup> modified first by Turesky *et al.* (1970)<sup>[9]</sup> and later by Lobene *et al.* (1982).<sup>[10]</sup> 1% erythrosine solution was used to disclose plaque, and the values were recorded on a 5-point scale at six sites per tooth.

Loe and Silness Gingival index (GI) was also evaluated, and only patients with GI scores of  $< 1$  at 40% of sites were included. All recordings were assessed by the same examiner. Thorough professional scaling and polishing were carried out 3 weeks prior to the initiation of trial. Participants were instructed to follow a meticulous oral hygiene protocol consisting of use of toothbrush and interdental cleaning. Participants were recalled at 1-week intervals for oral hygiene reinforcement and professional scaling where indicated. Baseline value of plaque score was recorded before the administration of mouthwash.

The enrolled participants were randomly allocated by computer generated random table method to either test group (Aloe vera mouth wash user,  $n = 30$ ), control group (2% chlorhexidine gluconate mouth wash user,  $n = 30$ ), placebo group (flavored distilled water).

The sample size of 90 participants of mouthwash study was calculated on the basis of following statistical evaluation:  $n = (Z_{\alpha/2} + Z_{\beta})^2 \times (p_1(1 - p_1) + p_2(1 - p_2)) / (p_1 - p_2)^2$ , where  $Z_{\alpha/2}$  is the critical value of the Normal distribution at  $\alpha/2$  (for a confidence level of 95%,  $\alpha$  is 0.05 and the critical value is 1.96),  $Z_{\beta}$  is the critical value of the normal distribution at

$\beta$  (for a power of 80%,  $\beta$  is 0.2 and the critical value is 0.84), and  $p_1$  (0.2% chlorhexidine gluconate mouthwash) and  $p_2$  (Aloe vera mouthwash) are the expected sample proportions of the two groups.

Aloe vera mouthwash consisted of pure Aloe vera Juice (Aloe vera Juice, Patanjali Ayurved Ltd, India). Composition of each 10 ml: 99.6% (w/v) Aloe vera juice; 0.02% (w/v) citric acid crystal; 0.02% (w/v) sodium benzoate crystal (preservative); Orange flavor (Q.S). 0.2% hexidine mouthwash (ICPA, Health Products, India).

Participants were instructed to refrain from regular oral hygiene practices i.e., tooth brushing or flossing, and instructed to use mouthwash only for next 4 days. Bottles of identical appearance with a volume of 150 ml of solution were dispensed to each participant who was blinded to the mouthwash received. A measure of 10 ml measure to ensure accuracy in dosage was given. All participants were given instructions for rinsing with 10 ml of the solution twice a day for duration of 1 min, once on rising, and once before bedtime. Ingestion of liquids or solids was permitted only after 2 h of rinsing.

This regimen was followed by each group for 4 days. PI scores were evaluated by the same trained examiner on the 5<sup>th</sup> day.

Mouthwashes were evaluated for side effects by subjective and objective criteria. Subjective criteria included: (1) Taste disturbance; (2) burning sensation; (3) dryness/soreness; (4) pruritis/itchiness; marked as 0 - absent; 1 - present. Objective criteria included (1) ulcer; (2) staining of teeth; (3) staining of tongue; (4) allergy; marked as 0 - absent; 1 - present.

### Statistical analysis

The means and standard deviation (mean  $\pm$  SD) values were determined for all PI scores. Intragroup comparison of plaque indices were done by analysis of variance (ANOVA) and post-hoc least significant difference (LSD) test. Side effects of the mouthwashes were assessed by subjective and objective criteria using a questionnaire. *P* value of 0.05 was considered to be significant.

### RESULTS

The protocol of the study was to be strictly followed by the participants, with no reported systemic side-effects. All the three groups mean plaque scores at baseline was  $2.69 \pm 0.055$  for control group and  $2.58 \pm 0.034$

for placebo, and after 4<sup>th</sup> day  $1.48 \pm 0.061$  for control group and  $2.79 \pm 0.037$  for placebo [Table 1]. Table 2 shows statistically significant values ( $P = 0.000$ ) in mean difference between pre and post-rinsing with *t* value of  $-24.63$  of the placebo group. Table 3 shows ANOVA test for difference between pre-rinsing PI scores between the three groups with an *f* value of 0.286 with no significant difference ( $P = 0.073$ ). Statistically significant value ( $P = 0.000$ ) with an *f* value of 6.676 were recorded in post-rinsing ANOVA test [Table 4]. Post-hoc LSD was used for multiple comparisons for post-rinsing PI score [Table 5]. Statistically significant difference was seen between control group and test group with placebo ( $P = 0.000$ ), although comparison between control group and test group was non-significant ( $P = 0.052$ ).

Subjective and objective evaluation of participants was done after 4 days of mouthwash usage. In the control group, staining was seen in 40%, taste in disturbance 25%, and burning sensation in 2% of subjects. Test group taste disturbance was seen in 4% of subjects. No staining was seen in the test group. There were no reported side effects in the placebo group.

### DISCUSSION

Periodontal health can be maintained by regular tooth-brushing, flossing, and rinsing with antibacterial agents containing mouthwashes. 0.2% chlorhexidine gluconate is commonly used in mouthwashes and irrigation. Stained tongue and teeth and alteration of taste sensation are common side effects which limit their use for prolonged period. Chlorhexidine also has documented effects on vital tissues such as cytotoxicity to periodontal ligament (PDL) cells, altered mitochondrial activity, and inhibition of protein synthesis.<sup>[11]</sup>

In recent times, there has been an increase in demand for alternative medicine.<sup>[12,13]</sup> Medicinal plants,

**Table 1: Mean value of plaque index score at baseline and after 4<sup>th</sup> day in different groups**

Group		Mean	Standard deviation	Standard error mean
CG	Base line	2.6940	0.05581	0.01019
	After 4 <sup>th</sup> day	1.4877	0.06191	0.01130
TG	Base line	2.8283	0.06449	0.01177
	After 4 <sup>th</sup> day	1.6047	0.04329	0.00790
Placebo	Base line	2.5850	0.03401	0.00621
	After 4 <sup>th</sup> day	2.7980	0.03718	0.00679

CG=Control group, TG=Test group

**Table 2: t-values for pre and post-rinsing plaque index scores of the groups**

Groups	Mean differences	Standard deviation	Standard error mean	t	df	Sig. (two-tailed)
CG	1.20633	0.07476	0.01365	88.377	29	0.000
TG	1.22367	0.08612	0.01572	77.823	29	0.000
Placebo	-0.21300	0.04735	0.00865	-24.637	29	0.000

CG=Control group, TG=Test group

**Table 3: Analysis of variance for pre-rinsing plaque index scores of different groups**

Variable	Source of variables	Sum of squares	df	Mean square	F	Sig.
Pre-rinsing	Between groups	0.891	2	0.446	0.286	0.073
	Within groups	0.244	87	0.003		
	Total	1.136	89			

**Table 4: Analysis of variance for post-rinsing plaque index scores of different groups**

Variable	Source of variables	Sum of squares	df	Mean square	F	Sig.
Post-rinsing	Between groups	31.547	2	15.774	6.676	0.000
	Within groups	0.206	87	0.002		
	Total	31.753	89			

such as Aloe vera, have natural phytochemicals and have proven to be better than synthetic drugs.<sup>[14,15]</sup> Studies by Khalessi *et al.*<sup>[16]</sup> and Parwani *et al.*<sup>[17]</sup> had suggested the better efficacy of herbal mouthwashes in maintaining plaque and gingival status. *In vitro* study on antimicrobial efficacy exposing supragingival plaque have found to have maximum effect at 100% concentration of Aloe vera.<sup>[18]</sup> In our study, 100% concentration of Aloe vera was used.

The present study is in compliance with the ideal mouth rinse protocol requirements given by Barnett.<sup>[19]</sup> There was a significant decrease in PI scores at the 4<sup>th</sup> day in the control and test group. Chlorhexidine gluconate is effective against assorted microorganisms, such as Gram-positive organisms, Gram-negative organisms, yeasts, viruses, and fungi is known. However, there are few studies, mainly *in vivo* studies on microorganisms such as *Streptococcus*, *Actinomyces viscosus*, and *Candida albicans* to suggest antimicrobial effect of Aloe vera.<sup>[20]</sup> Thus, the low plaque score in our study shows antimicrobial efficacy of Aloe vera, as seen in the study by Chandrahas *et al.*<sup>[21]</sup>

Aloe vera contains various anti-inflammatory agents such as carboxypeptidase, which reduce prostaglandin synthesis, magnesium lactate, which inhibits histidine decarboxylase preventing mast cell activity, sterols,

and lupeol as pain modulators.<sup>[22]</sup> Aloe vera also reduces edema by inhibiting matrix metalloproteinases blocking polymorphonuclear leucocyte (PMNs) release, cyclooxygenase, and lipo-oxygenase pathways. These activated PMNs in turn inhibit free oxygen radicals.<sup>[23]</sup> These suggest potential anti-inflammatory action of Aloe vera, which showed a decrease in the GI.<sup>[15,24]</sup>

Both the control and test groups showed significant reductions in plaque scores (chlorhexidine 46%, Aloe vera, 44%) which are consistent with previous studies. Aloe vera use was not associated with any side-effect, and post-rinsing PI scores between control group and test group were also not statistically significant. This suggests that Aloe vera has a similar potential as chlorhexidine as far as antiplaque activity is concerned.<sup>[25]</sup>

Shortcomings of this study are:

- A 4-day plaque regrowth model is used for the first time which is insufficient to evaluate gingival scores
- A cross-over design with wash-out period would have been more authenticating because it eliminates the bias of variable host response.

Therefore, future studies can be directed toward testing Aloe vera-based herbal mouthwash antiplaque and anti-gingivitis spectrum with a prolonged usage period.

## CONCLUSIONS

Within the limitations of this study, it can be concluded that Aloe vera mouth wash has an efficacy which is comparable to the antiplaque agent 0.2% chlorhexidine gluconate mouthwash. Considering the side effects associated with chlorhexidine, Aloe vera mouthwash can be considered as a viable alternative. However, studies (with a long-term rinsing period) should be carried out done to delineate the advantages and disadvantages associated with this herbal mouthwash.

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Nil.

**Table 5: Post-hoc for post-rinsing plaque index scores of different groups**

Dependent variable	(I) Group	(J) Group	Mean difference (I-J)	Standard error	Sig.
Post-rinsing	CG	TG	-0.11700*	0.01255	0.046
		Placebo	-1.31033*	0.01255	0.000
	TG	CG	0.11700*	0.01255	0.046
		Placebo	-1.19333*	0.01255	0.000
	Placebo	CG	1.31033*	0.01255	0.000
		TG	1.19333*	0.01255	0.000

N, Mean difference is significant at the 0.05 level, CG=Control group, TG=Test group

### Conflicts of interest

There are no conflicts of interest.

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