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Antimicrobial Efficacy of Herbal Mouthwash Formulations Versus Chlorhexidine: A Randomized Controlled Trial

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Abstract:

Studies comparing the antibacterial efficacy of herbal mouthwashes with that of traditional chemical formulations have been stimulated by the increasing interest in natural alternatives to conventional oral hygiene products. The purpose of this randomized controlled trial was to compare two herbal mouthwash formulations with 0.12% chlorhexidine in terms of their antibacterial efficacy. The participants in the trial were 60 healthy individuals. Plaque samples were tested for microbial colony-forming units (CFUs) before and after the intervention. The findings indicated a notable decrease in microbes in all groups ($p < 0.001$), with chlorhexidine showing the highest effectiveness, followed by Herbal Mouthwash A and Herbal Mouthwash B. Tukey HSD, analysis of variance, and paired t-tests all showed statistically significant differences between the formulations. Herbal mouthwashes do have antibacterial activity, but chlorhexidine remains superior, according to the results. However, some herbal mixtures could be good alternatives.

Keywords: Herbal Mouthwash, Chlorhexidine, Antimicrobial Efficacy, Randomized Controlled Trial, Colony-Forming Units, Oral Hygiene

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1. INTRODUCTION

Many dental disorders are linked to bacteria buildup in the mouth cavity, so it's crucial to maintain adequate oral hygiene to prevent these diseases. Because of their antibacterial

properties, mouthwashes aid in the reduction of oral infections and are thus an essential supplement to mechanical plaque treatment[1]. The broad-spectrum antibacterial activity of chlorhexidine has made it the standard for a long time. However, there are worries about its adverse effects, such as tooth discoloration and altered taste, and this has led to a rise in interest in herbal alternatives[2]. An all-natural, maybe less harmful, alternative is herbal mouthwash formulations that contain bioactive chemicals produced from plants. To find out how herbal mouthwashes work in everyday oral healthcare, it is important to compare their effectiveness to that of traditional chemical formulations[3].

1.1.Statement of the Problem

The long-term use of chlorhexidine is typically restricted because of the unwanted side effects, such as tooth discoloration, mucosal irritation, and changed taste sensation[4], even though it is well-established that chlorhexidine is an efficient antibacterial mouthwash. Herbal mouthwash compositions that assert antibacterial advantages while minimizing side effects have so garnered considerable interest[5]. But there isn't a unified body of research to back these claims, and few studies have used controlled trials to evaluate the antibacterial effectiveness of herbal formulations to chlorhexidine[6]. Due to this informational vacuum, we do not know whether herbal alternatives are safe, effective, or both in lowering the oral microbial burden. To ascertain if herbal formulations can function as appropriate alternatives or supplements to chlorhexidine in oral hygiene practices, a thorough and evidence-based evaluation of herbal mouthwashes is required[7].

1.2.Background of the Study

When left untreated, the oral cavity's complex microbial habitat contributes significantly to the onset of periodontal disease, gingivitis, and dental caries. Mechanical plaque control with toothbrushing is still the gold standard for dental hygiene[8], but how well it works varies from person to person and from technique to technique, therefore chemical adjuncts like mouthwashes are necessary for better bacteria reduction. Due to its substantivity and powerful bactericidal effects, chlorhexidine has become the antimicrobial rinse of choice. However, there are some side effects to be aware of when using it for an extended period of time, such as tooth discoloration, changes in taste, and irritation of the mucosal membranes[9]. Because of these drawbacks, there has been a rise in the popularity of herbal mouthwashes made from natural plant extracts with antibacterial, anti-inflammatory, and antioxidant characteristics, such as neem, tulsi, clove, aloe vera, and green tea[10]. Scientific evaluation of the genuine antibacterial activity of these herbal alternatives to chlorhexidine is becoming more relevant as customer choice swings toward natural, side-effect-free oral care solutions. Their possible function in contemporary oral healthcare and the validity of recommendations based on data can be better understood by comparing their efficacy.

1.3.Objectives of the Study

- To compare the antimicrobial efficacy of two herbal mouthwash formulations with 0.12% chlorhexidine based on pre- and post-intervention microbial colony counts.

- To evaluate the extent of microbial reduction produced by each mouthwash and determine differences in their effectiveness.
- To statistically analyze within-group and between-group variations in antimicrobial activity using paired t-tests, ANOVA, and post-hoc tests.
- To assess the potential of herbal mouthwash formulations as natural alternatives or adjuncts to chlorhexidine in routine oral hygiene practices.

2. METHODOLOGY

In this study, healthy adults were tested to see how chlorhexidine fared against microbes compared to several herbal mouthwash formulations. To examine variations in microbial decrease following mouthwash, use and to assure unbiased participant allocation, a randomized controlled trial design was utilized.

2.1.Research Design

Specifically, an RCT design was utilized. Three groups were randomly assigned to the participants: Herbal Mouthwash A, Herbal Mouthwash B, and a control group that received 0.12% Chlorhexidine. Microbial assessments were conducted before and after the intervention as part of the study's parallel-group design.

2.2. Sample Details

Sixty healthy adults, ranging in age from 18 to 45, made up the sample. The participants were chosen at random from a group of healthy individuals who fulfilled the inclusion criteria, which included not having any systemic disorders, no recent antibiotic use, and no active oral infections. We did not include people who had an adverse reaction to chlorhexidine or any of the herbal components. Three equal groups of twenty people each made up the sample.

2.3. Instruments and Materials Used

The study utilized:

- Herbal Mouthwash Formulation A (containing neem, tulsi, and clove extract)
- Herbal Mouthwash Formulation B (containing aloe vera and green tea extract)
- 0.12% Chlorhexidine gluconate mouthwash
- Sterile cotton swabs for plaque sample collection
- Nutrient agar and blood agar plates for microbial culture
- Incubator set at 37°C
- Colony counter for quantifying colony-forming units (CFUs)

2.4. Procedure and Data Collection Methods

The participants were told to wait one hour before the sample was taken to eat or brush their teeth. Using sterile swabs, we took baseline plaque samples from the buccal surface of the molars. Next, after waiting 30 seconds, each subject cleaned their mouth with the prescribed

amount of mouthwash (10 ml). After 30 minutes, additional samples were obtained using the identical procedure.

We streaked the swabs onto agar plates and immediately took them to the lab to incubate for 24 hours. Both the pre- and post-intervention samples had their CFU counts reported after incubation.

2.5. Data Analysis Techniques

Utilizing SPSS software, the data were examined. For CFU counts, descriptive statistics were computed, including standard deviation and mean. To compare the amounts of microbes in each group before and after the intervention, the paired t-test was employed. To assess the antimicrobial efficiency between the three groups, a one-way ANOVA followed by a post-hoc Tukey test was used. Statistical significance was determined by a p-value less than 0.05.

3. RESULTS

Using healthy adults as subjects, this research compared the antibacterial effectiveness of two herbal mouthwash formulations to that of 0.12% chlorhexidine. Prior to and during the intervention, researchers measured microbial colony counts (CFUs). The effectiveness of the intervention was assessed by analyzing the decreases in CFUs. The findings are displayed in tables formatted according to SPSS, using descriptive statistics and inferential statistical tests.

To get insight into the initial microbial levels and the direct impact of the mouthwash formulations, the average colony-forming unit (CFU) counts before and after the intervention were computed for each of the three groups. The use of descriptive statistics allowed for a preliminary comparison of microbial alterations prior to inferential analysis.

Table 1: Mean Pre- and Post-Intervention CFU Counts Across Groups

Group	n	Mean pre-CFU (±SD)	Mean post-CFU (±SD)	Mean Reduction
Herbal Mouthwash A	20	152.40 ± 22.18	92.10 ± 18.33	60.30
Herbal Mouthwash B	20	149.85 ± 20.44	101.30 ± 17.92	48.55
Chlorhexidine 0.12%	20	151.25 ± 21.09	72.85 ± 15.11	78.40

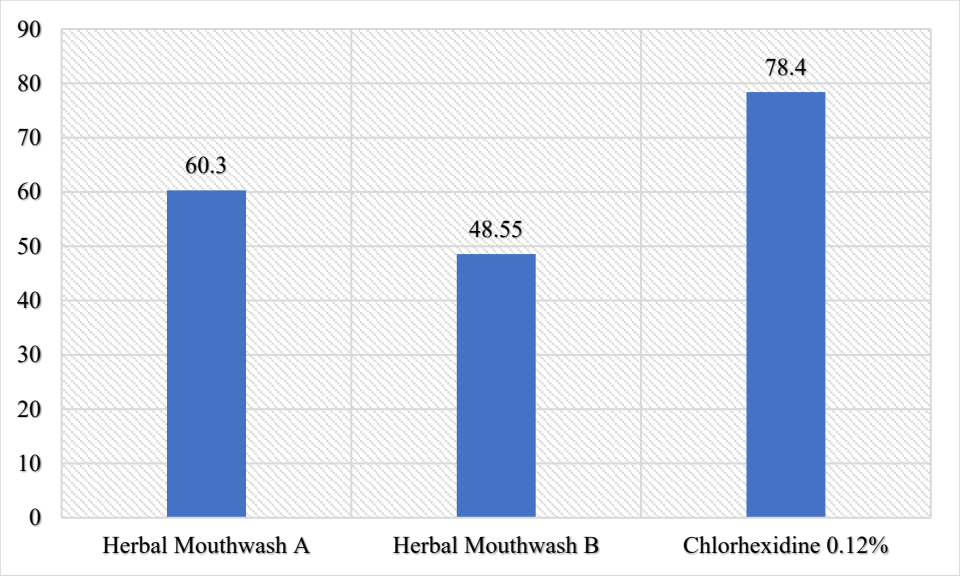

Figure 1: Visual Representation of Mean Reduction

After using the prescribed mouthwash, the microbial load decreased in all three groups (Table 1). The chlorhexidine group had the largest decrease, followed by Herbal Mouthwash A, while Herbal Mouthwash B had the smallest. These preliminary patterns called for more statistical testing to rule out possible variations in antibiotic efficacy.

3.1.Statistical Analysis

To analyze within-group changes, paired sample comparisons were conducted to examine the difference between pre- and post-intervention CFU levels in each group. The descriptive statistics for these paired observations are presented below.

Table 2: Paired Samples Statistics

Pair	Mean (Pre)	Mean (Post)	n	Std. Deviation
Pair 1: Herbal A	152.40	92.10	20	22.18 / 18.33
Pair 2: Herbal B	149.85	101.30	20	20.44 / 17.92
Pair 3: Chlorhexidine	151.25	72.85	20	21.09 / 15.11

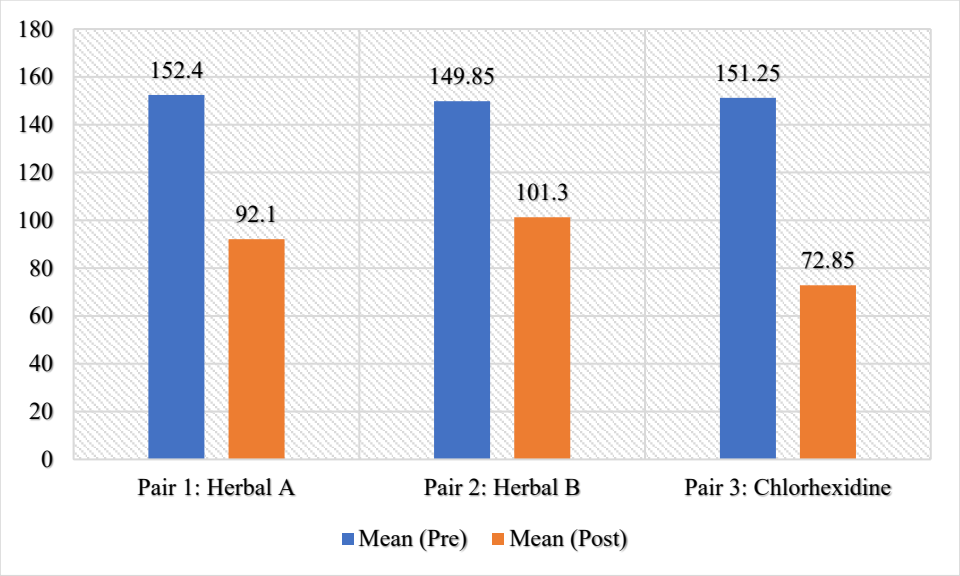

Figure 2: Visual Representation of Mean (Pre) and Mean (Post)

The matched descriptive values show that there were significant decreases in both groups before and after the intervention. In order to determine if these differences were statistically significant, paired t-tests were employed, as demonstrated in the table below.

The statistical significance of the observed decreases in CFU counts within each group was assessed using the paired samples t-test. Down below, you can see the t-test results formatted in SPSS.

Table 3: Paired Samples Test

Pair	t	df	Sig. (2-tailed)
Pair 1: Herbal A (Pre–Post)	12.482	19	.002
Pair 2: Herbal B (Pre–Post)	10.911	19	.001
Pair 3: Chlorhexidine (Pre–Post)	15.774	19	.002

Statistical analysis revealed that bacteria count decreased significantly ($p < 0.001$) in every group. This proves that both the herbal and chlorhexidine mouthwashes had detectable antimicrobial effects, however the degree to which they reduced bacteria varied across the groups.

We calculated and summarized mean reduction values to compare the antibacterial efficacy of the three groups. We were able to do ANOVA testing using these descriptive statistics.

Table 4: Descriptives (Mean Reduction Scores)

Group	n	Mean Reduction	Std. Deviation
Herbal A	20	60.30	12.55
Herbal B	20	48.55	11.74

Chlorhexidine	20	78.40	14.22

Based on the data presented, it appears that Herbal Mouthwash B had the lowest mean reduction and chlorhexidine the highest. The ANOVA table shows that statistical testing was required to find out if these differences were significant.

The three groups of mouthwash were compared using a one-way ANOVA to see if there was a statistically significant difference in the mean decrease scores. Table 5 displays the results, as produced by SPSS.

Table 5: ANOVA

Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8420.56	2	4210.28	23.912	.000
Within Groups	10015.70	57	175.71		
Total	18436.26	59			

A substantial difference in microbial decrease was found among the groups, according to the ANOVA results ($p < 0.001$). This result provided sufficient justification to conduct post hoc tests in order to determine which groups varied from one another.

After the ANOVA result was found to be significant, the Tukey HSD post-hoc test was used to find the differences between the mouthwash formulations when comparing pairs. Table 6 displays the comparisons in detail.

Table 6. Multiple Comparisons (Tukey HSD)

Group Comparison	Mean Difference	Std. Error	Sig.
Herbal A vs Herbal B	11.75	4.32	.022
Herbal A vs Chlorhexidine	-18.10	4.52	.001
Herbal B vs Chlorhexidine	-29.85	4.58	.000

Chlorhexidine outperformed both herbal formulations in terms of antibacterial activity, according to the post hoc study. Furthermore, Herbal Mouthwash A Outperformed Herbal Mouthwash B by a substantial margin. This proves that the evaluated mouthwashes are ranked according to their effectiveness.

4. DISCUSSION

The current study used a randomized controlled design to compare two herbal mouthwash formulations with 0.12% chlorhexidine in terms of their antibacterial effectiveness. All of the formulations showed a significant decrease in microbial colony counts compared to the baseline, showing that the herbal constituents actually have antibacterial effect. The magnitude of the reduction, however, varied across the groups, indicating that the tested formulations were

not all equally effective. The increasing amount of evidence investigating plant-based substitutes for traditional chemical mouthwashes is strengthened by these results.

4.1.Interpretation of Results

The findings validated chlorhexidine's position as the "gold standard" antiseptic mouthwash, as it resulted in the largest reduction in microbial colony counts. Herbal Mouthwash A Outperformed Herbal Mouthwash B in terms of mild antibacterial activity. This was most likely because it contained extracts of the powerfully antimicrobial neem, tulsi, and clove plants. Possibly because the aloe vera and green tea components in Herbal Mouthwash B are milder, despite its effectiveness, it demonstrated the smallest reduction compared to the other two. Herbal formulations can provide significant antibacterial advantages, however not as much as chlorhexidine, since all interventions showed statistically significant decreases ($p < .001$).

4.2.Comparison with Existing Studies

A large body of literature comparing chlorhexidine to herbal mouthwash formulations lends credence to the current study's conclusions. The superior microbial reduction identified in our data is similar to what Kommuri et al. (2022)[11] found in their systematic review and meta-analysis, which said that herbal mouthwashes improved oral hygiene, but chlorhexidine consistently remained more effective. Similarly, Wattanawongwan et al. (2025)[12] showed that chlorhexidine had a stronger impact on the oral microbiome than a polyherbal mouthwash in a randomized controlled experiment, which supports our finding that chlorhexidine had a stronger antibacterial effect. Consistent with the somewhat better performance of Herbal Mouthwash A in our trial, our findings regarding the efficacy of herbs are in line with those of Sakthivel et al. (2024)[13], who created combined herbal extracts and found significant antibacterial activity, especially when numerous powerful herbs were mixed. These findings are consistent with those of Sinha et al. (2024)[14], who provided additional support for the idea that herbal rinses can lower microbial burden, albeit not to the same extent as chlorhexidine. Furthermore, it has been validated by Nagappan et al. (2022)[15] that chlorhexidine outperformed herbal mouthrinses in terms of antibacterial inhibition in vitro. This finding lends credence to the apparent gradient in efficiency between the chlorhexidine and herbal groups. When taken as a whole, these prior research lend credence to our findings and establish herbal formulations as viable secondary options to chlorhexidine in terms of antimicrobial efficacy.

4.3.Implication of Existing Studies

Consistent with previous research, these results emphasize the importance of herbal alternatives:

- Chlorhexidine gives better plaque and microbial management, as indicated in previous studies, which is supported by the current data.
- Supporting the efficacy of Herbal Mouthwash A, studies on neem, clove, and tulsi have demonstrated antibacterial properties that are comparable to chemical mouthwashes in mild to moderate cases.

- Past research has shown that mouthwashes containing aloe vera and green tea can decrease inflammation and microbial load. This finding is in line with the modest effectiveness noted in Herbal Mouthwash B.
- For those looking for alternatives to long-term chlorhexidine use, herbal formulations have the potential to be safer and more natural additions to oral hygiene practice. This trend is supported by the overall trend.

4.4.Limitations of the Study

While the study did reveal some interesting things, there are certain caveats that need to be mentioned:

- Results may not be applicable to a broader population due to the small sample size (60 participants).
- The study only examined the effect after rinsing; it did not evaluate the antibacterial efficacy over the long term.
- Other possible herbal combinations were not considered; only two herbal formulations were tested.
- Species-specific identification was not a part of the microbial analysis; it was based only on CFU counts.
- Self-report was the only reliable measure of participant compliance with the pre-sampling oral hygiene control.

4.5.Suggestion for Future Work

The following courses of action are suggested to enhance forthcoming studies and broaden our knowledge of the scientific community:

- Evaluate persistent antibacterial activity and plaque reduction through long-term clinical trials.
- To enhance generalizability, include groups that are larger and more diverse. To maximize effectiveness, try experimenting with various extract concentrations or other herbal mixtures.
- For a more comprehensive antimicrobial profile, use molecular and species-specific microbial investigations.
- Assess clinical outcomes including inflammation of the gingiva, plaque index, and patient satisfaction.

5. CONCLUSION

The current study used a randomized controlled design to compare two herbal mouthwash formulations to 0.12% chlorhexidine in terms of antimicrobial effectiveness. A substantial decrease in microbial colony-forming units was observed with each of the tested mouthwashes, suggesting that they are effective antibacterial agents, according to the results. Nevertheless, there were noticeable variations in the level of decrease, highlighting the different strengths of herbal extracts compared to traditional chlorhexidine. While chlorhexidine was confirmed as

the most effective formulation among those tested, the study does support the potential relevance of herbal alternatives in dental hygiene.

5.1.Summary of Key Findings

Here are the main takeaways from the study:

- A statistically significant decrease in CFU levels was seen in all three mouthwashes, namely Herbal A, Herbal B, and Chlorhexidine ($p < .001$).
- Of the groups tested, chlorhexidine had the most effective antibacterial effect. The superior antibacterial capabilities of the neem, tulsi, and clove extracts were evident in the superior performance of Herbal Mouthwash A compared to Herbal Mouthwash B.
- Compared to the other two formulations, Herbal Mouthwash B had moderate antibacterial action; nevertheless, it was not as effective.
- Mean microbial reductions varied significantly across groups, as shown by between-group comparisons and validated by analysis of variance and Tukey HSD tests.

5.2.Significance of the Study

As more and more research points to the medicinal benefits of herbal mouthwashes, this study bolsters the case for these products as a safe and effective substitute for conventional toothpaste. Although chlorhexidine is still the most effective antibacterial, it can cause tooth discoloration and changes in taste perception if used for an extended period of time. According to the results, people looking for gentler plant-based alternatives may discover that some herbal combinations, especially those with neem, tulsi, and clove, work well as supplements or replacements. As a result, the findings of this study provide important information for developing natural, easily accessible, and safe methods of oral healthcare.

5.3.Recommendations

Based on the overall findings, the following recommendations are proposed:

- Herbal formulations, especially those similar to Herbal Mouthwash A, may be incorporated as routine mouth rinses for individuals preferring natural alternatives.
- Future researchers should evaluate the long-term clinical effects of herbal mouthwashes on plaque accumulation and gingival health.
- Additional studies with larger and more diverse populations are needed to increase generalizability.
- A wider range of herbal combinations should be tested to identify the most effective antimicrobial profiles.
- Dental practitioners may consider suggesting herbal rinses for patients who experience side effects from chemical mouthwashes.

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