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Comparative Evaluation of Anti-Gingivitis and Anti-Plaque Efficacy of Tulsi and Pomegranate Extract Mouthwashes: A Clinical Study

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Abstract

Background: This study is aimed to compare the effectiveness of mouthwashes containing Tulsi extract, Pomegranate peel extract, Chlorhexidine, and placebo (Distilled water) when used in combination with scaling and root planing (SRP) therapy for managing gingivitis and plaque.

Methodology: A randomized controlled trial design was employed, recruiting 120 participants who were randomly allocated into four groups: SRP + Tulsi extract mouthwash, SRP + Pomegranate peel extract mouthwash, SRP + 0.2% Chlorhexidine mouthwash, and SRP + Placebo mouthwash (Distilled water). Plaque index, gingival index, and *P. gingivalis* colony forming units were assessed at baseline and after 21 days.

Results: Intragroup comparisons revealed a statistically significant reduction in mean plaque index, gingival index and *P. gingivalis* colony forming units in all groups from baseline to 21 days. Intergroup comparisons demonstrated significant differences in mean plaque index, gingival index and *P. gingivalis* colony forming units among the groups except placebo mouthwash group.

Conclusion: The findings suggest that mouthwashes containing Tulsi extract and Pomegranate peel extract when used in combination with SRP therapy, exhibit promising anti-gingivitis and anti-plaque efficacy comparable to Chlorhexidine. These herbal extracts may serve as effective alternatives for managing gingivitis and plaque in dental care.

Keywords: Gingivitis, plaque, mouthwash, Tulsi extract, pomegranate peel extract, chlorhexidine, clinical study

1. Introduction

Dental plaque is the primary etiological agent responsible for periodontal diseases. Early stages of gingivitis can be reversed by meticulous plaque control through oral hygiene procedures. The majority of the population may not perform mechanical plaque removal properly [1]. Thus, antimicrobial mouth rinses that improve daily home care may provide an effective way of removing or controlling bacterial plaque to limit gingivitis and periodontitis. Chemotherapeutic mouth rinses are an effective adjunct to regular brushing and flossing for patients with gingivitis, thus providing a clinically significant plaque reduction and treatment of gingivitis [2]. Chlorhexidine mouthwashes have their own side effects [3]. Herbal mouthwashes have no side effects and are safe to use, have become a viable option for mechanical therapy.

Ayurveda is a science of life. Ayurveda is an ancient Indian medical science, origin of which can be traced back to Vedas. "Dant-shashtra" is a branch of Ayurveda, which is the modern-day dentistry. It started with chewing sticks and has come to the age of mouthwashes. There is an increased interest in finding safe herbal alternatives to commercial mouthwashes [4].

Tulsi and pomegranate are two of the most commonly used herbs in dentistry. Tulsi has been used for thousands of years in Ayurveda for its diverse healing properties. It is also known as the "Queen of Herbs", "Incomparable one", "Elixir of life", "Adaptogen". One may consider the pomegranate to be "A pharmacy unto itself" [5].

2. Literature Review

Dabholkar *et al.* (2016) investigated the antibacterial activity of pomegranate-infused mouthwash on dental biofilm-forming microorganisms [6]. Three types of mouthwash were used: Group C received a mouthwash containing pomegranates (Life-extension), Group B received an herbal mouthwash (Hiora), and Group A received a mouthwash containing chlorhexidine (Hexidine). Each mouthwash came in five different concentrations. Researchers selected reference strains of *S. mutans*, *S. salivarius*, and *A. a.* as the colonizers when it came time to create dental biofilms. After the incubation time, the inhibitory zone widths were assessed. Hiora and Pomegranate mouthwashes showed varying degrees of inhibition, in contrast to Chlorhexidine, which demonstrated a concentration-dependent inhibition zone for all three organisms. When evaluated at different concentrations, the statistical analysis showed significant differences between the mouthwash groups, suggesting that all three of the mouthwashes have antibacterial qualities against microorganisms that can form biofilms.

Singla *et al.* (2018) [7] investigated the effects of mouthwashes containing extracts from guava, pomegranate, and grape seeds on salivary streptococci levels over a 48-hour and 7-day period, using two applications per day. Forty school-age children were randomly assigned to one of four groups ($n = 10$ in each experimental group). Participants received oral rinses consisting of distilled water (control), pomegranate, grape seed, or guava twice a day. Salivary samples were inoculated onto blood agar media after 48 hours and 7 days to quantify the colony-forming units/ml (CFU/ml) of salivary streptococci. The results illustrated that the aqueous extracts of the selected herbal plants efficiently targeted oral streptococci. The guava, pomegranate, and grape seed extracts may be beneficial as all-natural substitutes for traditional mouth bacteria management techniques.

Gattani *et al.* (2019) [8] examined ultrasonic scaling and irrigation for the treatment of chronic gingivitis using 2% *Occimum sanctum* extract, 0.2% chlorhexidine, and distilled water. Forty-five participants, ages twenty-five to sixty-five, were randomly assigned to three groups: Group A received ultrasonic scaling with 2% *Occimum sanctum* extract, Group B received 0.2% chlorhexidine, and Group C received distilled water (control). The Sulcular Bleeding Index, Plaque Index, and Gingival Index were used in the baseline, 7-day, and 21-day evaluations. Plaque, gingival scores, and sulcular hemorrhage were all considerably lower between groups as compared to within groups. However, there were no appreciable changes between Groups A and B. Furthermore, Groups A and B (containing Tulsi extract and chlorhexidine, respectively) demonstrated significantly less gingival irritation in comparison to Group C (containing distilled water).

Richard *et al.* (2019) [9] investigated the efficacy of Tulsi, Triphala, and a combination of the two types of mouthwash in decreasing gingival and plaque scores over chlorhexidine mouthwash. A randomized parallel-arm clinical trial had 175 participants who met the inclusion and exclusion criteria. Of these, 35 were divided into five groups: chlorhexidine, Triphala, Tulsi, combination, or placebo. During the 30-day experiment, participants were expected to swish 10 milliliters of the chosen mouthwash around in their mouths for 45 seconds twice daily. Tulsi and Triphala mouthwash considerably lowered gingival ratings when compared to chlorhexidine and placebo, and Tulsi also dramatically reduced plaque scores. Tulsi and Triphala mouthwashes were also proven to be efficient in reducing gingivitis and plaque over time while remaining safe to use. The mouthwashes containing 0.6% Triphala and 0.4% Tulsi were more effective than chlorhexidine mouthwash in lowering gingival scores and plaque, implying that these alternatives may be advantageous in oral hygiene.

Trilochansai *et al.* (2023) [10] conducted a double-blind randomized clinical trial to assess the efficacy of Triphala, Tulsi extract, and chlorhexidine gluconate in managing gingivitis. Three mouthwash options-Triphala, Tulsi, or chlorhexidine-were distributed to a total of sixty participants. The findings demonstrated that the degree of plaque and gingivitis reduction achieved by both chlorhexidine and the *Ocimum sanctum* (Tulsi extract) mouthwash was comparable. Comparing the Triphala and Tulsi groups to the chlorhexidine group, the latter depicted a significant decrease in plaque indices and gingival bleeding after 15 and 30 days, respectively. While chlorhexidine remained the gold standard for treating plaque and gingivitis, more recent and promising options such as Triphala and Tulsi showed significant potential.

Hussin *et al.* (2024) [11] investigated the medicinal potential of natural herbs in dentistry, aiming to determine the varied varieties, their active components, and the dental applications for each. Extensive keyword searching across WoS, Pubmed, and Scopus was done to find relevant material. According to the data, 512 papers were published between 2015 and 2023. When *in vitro* tests and clinical trials were the primary inclusion criteria, a total of 128 papers were deemed relevant. Of them, 42 were clinical trials and 86 were *in-vitro* investigations. The majority of focus was paid to the antibacterial properties (50.0%), whereas pulp-capping and obturation material received less attention (0.8%). Many common medical conditions, such as gingivitis, denture stomatitis, and oral candidiasis, have shown promise when treated with herbal remedies. Herbs that help treat a range of dental issues include propolis, ginger, green tea, and extract from *Rhizoma imperatae*. Conversely, the mouthwash containing *Pistacia atlantica* extract showed positive antibacterial and antiplaque properties.

TABLE 1: COMPARATIVE SUMMARY OF HERBAL AND CHEMICAL MOUTHWASHES IN DENTAL CARE

Study	Intervention	Sample Size	Main Findings
Dabholkar <i>et al.</i> (2016) [6]	Pomegranate, Chlorhexidine mouthwashes	-	All mouthwashes exhibited antimicrobial activity against biofilm-forming organisms at varying concentrations. Chlorhexidine showed significant inhibition across concentrations. Herbal mouthwashes also showed inhibition, suggesting potential as antimicrobial agents.
Singla <i>et al.</i> (2018) [7]	Pomegranate, Grape seed, Guava, Distilled water mouthwashes	40 children	Aqueous extracts of pomegranate, grape seed, and guava exhibited antibacterial efficacy against oral streptococci. Potential for natural extracts as alternatives for controlling oral bacteria.
Gattani <i>et al.</i> (2019) [8]	Tulsi, Chlorhexidine, Distilled water mouthwashes	45 subjects	Significant reductions in plaque, sulcular bleeding, and gingival scores for all groups. No significant differences between Tulsi and chlorhexidine groups. Tulsi and chlorhexidine exhibited significantly reduced gingival inflammation compared to distilled water.
Richard <i>et al.</i> (2019) [9]	Tulsi, Triphala, Chlorhexidine mouthwashes	175 participants	Significant reduction in plaque scores with Tulsi mouthwash. Triphala mouthwash significantly reduced gingival scores compared to chlorhexidine and placebo. Both Tulsi and Triphala mouthwashes demonstrated sustained efficacy without adverse effects.
Trilochansai <i>et al.</i> (2023) [10]	Triphala, Tulsi, Chlorhexidine mouthwashes	60 individuals	Tulsi mouthwash is just as good at preventing plaque and gingivitis as chlorhexidine. Significant reductions in gingival bleeding and plaque indices with Triphala and Tulsi compared to chlorhexidine over 15 and 30 days. Triphala and Tulsi showed promise as alternatives for plaque and gingivitis management.
Hussin <i>et al.</i> (2024) [11]	Various herbal mouthwashes	512 articles	Predominant focus on antimicrobial properties (50.0%). Herbs like propolis, green tea, ginger, and <i>Rhizoma imperatae</i> extract effectively treat dental conditions. <i>Pistacia atlantica</i> extract mouthwash effective against dental plaque bacteria and subgingival microorganisms.
Boyapati <i>et al.</i> (2024) [12]	Probiotic, Aloe vera, Povidone-iodine, Chlorhexidine mouthwashes	40 patients	Significant decrease in marginal plaque index (MPI) and bleeding on interdental brushing (BOIB) scores in all groups. Probiotic mouthwash showed efficacy comparable to chlorhexidine in treating gingivitis. Herbal and povidone-iodine groups showed similar efficacy from baseline to day 28.

3. Methodology

3.1 Study Design: This study utilized a randomized controlled trial design to assess the comparative efficacy of mouthwashes containing Tulsi extract, Pomegranate peel extract, Chlorhexidine, and a placebo (Distilled water) in combination with scaling and root planing.

3.2 Participants: After recruitment, a total of 120 individuals were divided randomly into four groups of thirty each.

3.3 Interventions:

1. Group I received SRP along with Tulsi extract mouthwash.
2. Group II received SRP along with Pomegranate peel extract mouthwash.
3. Group III received SRP along with 0.2% Chlorhexidine mouthwash.
4. Group IV received SRP along with placebo mouthwash (Distilled water).

3.4 Assessments

1. **Plaque Index Assessment:** Plaque index is a standardized method for quantifying the amount of dental plaque present on tooth surfaces. In this study, plaque index assessment involved visually examining the teeth and assigning a score to each tooth surface based on the amount of plaque present. This assessment was conducted at baseline (Visit 1) and after 21 days (Visit 2) to evaluate changes in plaque accumulation over time in response to the different mouthwash interventions.
2. **Gingival Index Assessment:** The gingival index is a metric for gingival tissue irritation. Ratings based on features such as colour, shape, and bleeding upon probing are obtained by visual inspection of the gingiva. Similar to the plaque index assessment, the gingival index assessment was conducted at baseline and after 21 days to evaluate changes in gingival health following the interventions.
3. ***P. gingivalis* Colony Forming Units Assessment:** *P. gingivalis* is a bacteria associated with periodontal disease. Assessing *P. gingivalis* colony forming units involves collecting samples from the oral cavity, culturing them in a laboratory setting, and counting the number of bacterial colonies formed. This assessment provides quantitative data on the presence and abundance of *P. gingivalis* in the oral microbiota. Similar to the plaque and gingival index assessments, this assessment was conducted at baseline and after 21 days to evaluate changes in *P. gingivalis* levels in response to the interventions.

3.5 Statistical analysis

Statistical analysis was performed using ANOVA to compare mean values of plaque index, gingival index, and *P. gingivalis* colony forming units among the four intervention groups at baseline and after 21 days. Post hoc Bonferroni tests were conducted to identify specific group differences.

4. Result

The present study included 120 patients of moderate chronic gingivitis, in the age group of 15-45. The patients were randomly divided into four groups. Plaque index, Gingival index and subgingival plaque samples were taken at baseline and 21 days.

Plaque index showed a significant decrease from baseline to 21 days in all the groups.

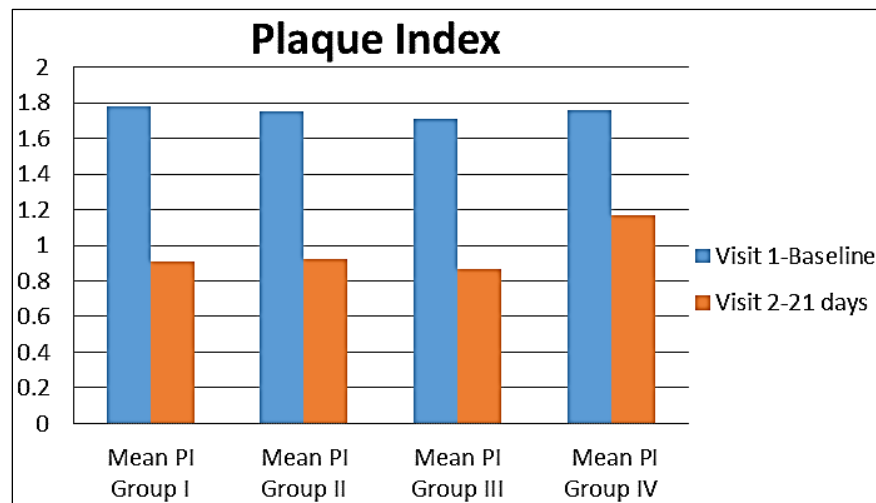
The mean decrease in plaque scores of Tulsi group was from 1.78 ± 0.44 at baseline to 0.87 ± 0.18 at 21 days. The pomegranate group showed mean reduction from 1.75 ± 0.37 at baseline to 0.92 ± 0.15 at 21 days. The chlorhexidine group had mean reduction from 1.71 ± 0.33 at baseline to 0.87 ± 0.18 at 21 days. Placebo group also showed mean reduction from 1.76 ± 0.42 at baseline to 1.17 ± 0.21 at 21 days.

Post hoc analysis showed a statistically significant difference between Tulsi extract mouthwash and Placebo mouthwash group, Pomegranate peel extract mouthwash group and placebo mouthwash group, chlorhexidine mouthwash group and placebo mouthwash group. Between Tulsi extract mouthwash group, Pomegranate Peel extract mouthwash group and Chlorhexidine mouthwash group, no statistical significance was found. (Table 2)

TABLE 2: INTERGROUP COMPARISON OF MEAN PLAQUE INDEX AMONG GROUP I, GROUP II, GROUP III AND GROUP IV

Parameter	Visits	Group I (N=30)	Group II (N=30)	Group III (N=30)	Group IV (N=30)	P-value (ANOVA)	Post hoc (Bonferroni test)					
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD		Grp I vs Grp II	Grp II vs Grp III	Grp III vs Grp IV	Grp I vs Grp III	Grp I vs Grp IV	Grp II vs Grp IV
Plaque Index	Visit 1 (Baseline)	1.78 ± 0.44	1.75 ± 0.37	1.71 ± 0.33	1.76 ± 0.42	0.642	NS	NS	NS	NS	NS	NS
	Visit 2 (21 Days)	0.87 ± 0.18	0.92 ± 0.15	0.87 ± 0.18	1.17 ± 0.21	<0.001	NS	NS	<0.001	<0.001	NS	<0.001

Anova



4.2 Gingival Index

Gingival index showed a significant decrease from baseline to 21 days in all the groups.

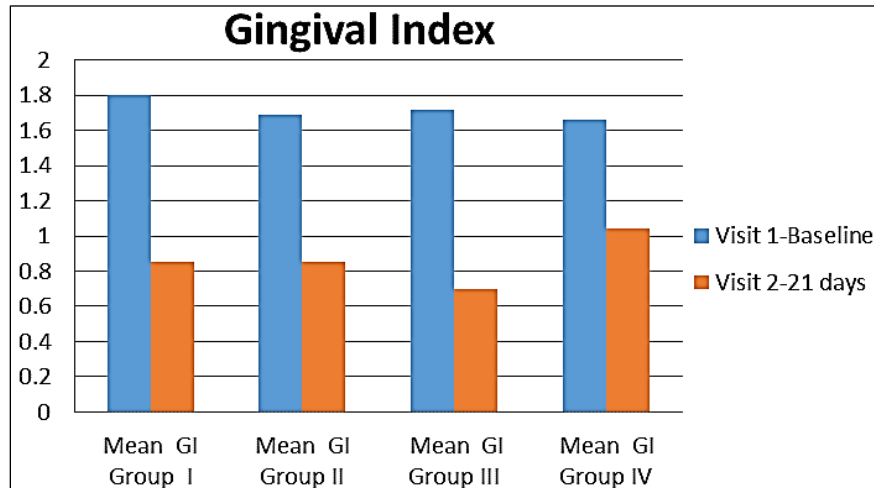
The mean decrease in gingival scores of Tulsi group was from 1.80 ± 0.15 at baseline to 0.85 ± 0.24 at 21 days. The pomegranate group showed mean reduction from 1.69 ± 0.22 at baseline to 0.85 ± 0.18 at 21 days. The chlorhexidine group had mean reduction from 1.72 ± 0.17 at baseline to 0.70 ± 0.21 at 21 days. Placebo group also showed mean reduction from 1.66 ± 0.21 at baseline to 1.04 ± 0.22 at 21 days.

Post hoc analysis showed a statistically significant difference between Tulsi extract mouthwash and Placebo mouthwash group, Pomegranate peel extract mouthwash group and placebo mouthwash group, chlorhexidine mouthwash group and placebo mouthwash group. Between Tulsi extract mouthwash group, Pomegranate Peel extract mouthwash group and Chlorhexidine mouthwash group, no statistical significance was found. (Table 3)

TABLE 3: INTERGROUP COMPARISON OF MEAN GINGIVAL INDEX AMONG GROUP I, GROUP II, GROUP III AND GROUP IV

Parameter	Visits	Group I (N=30) Mean $\pm$ SD	Group II (N=30) Mean $\pm$ SD	Group III (N=30) Mean $\pm$ SD	Group IV (N=30) Mean $\pm$ SD	P-value (ANOVA)	Post hoc (Bonferroni test)					
							Grp I vs Grp II	Grp II vs Grp III	Grp III vs Grp IV	Grp IV vs Grp I	Grp I vs Grp III	Grp II vs Grp IV
Gingival Index	Visit1 (Baseline)	1.80 $\pm$ 0.15	1.69 $\pm$ 0.22	1.72 $\pm$ 0.17	1.66 $\pm$ 0.21	0.742	NS	NS	NS	NS	NS	NS
	Visit 2 (21 Days)	0.85 $\pm$ 0.24	0.85 $\pm$ 0.18	0.70 $\pm$ 0.21	1.04 $\pm$ 0.22	<0.001	NS	NS	<0.001	<0.001	NS	<0.001

Anova

4.3 *P. gingivalis* colony forming units in all patients

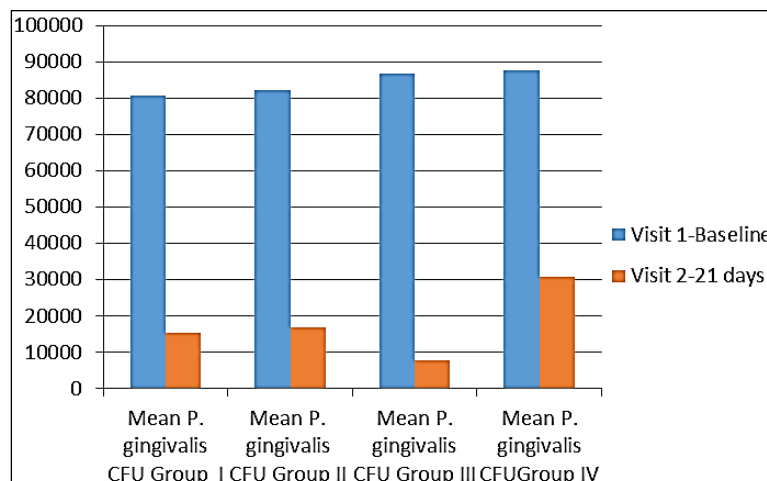
P. gingivalis colony forming units showed a significant decrease from baseline to 21 days in all the groups. The mean decrease in *P. gingivalis* colony forming units in Tulsi group was from 80800.00±11121.27 at baseline to 15266.66±4282.54 at 21 days. The pomegranate group showed mean reduction from 82166.66±17290.78 at baseline to 16866.66±3875.06 at 21 days. The chlorhexidine group had mean reduction from 86566.66±18222.16 at baseline to 7633.33±2399.47 at 21 days. Placebo group also showed mean reduction from 87766.66±14977.41 at baseline to 30633.33±6990.05 at 21 days.

Post hoc analysis showed a statistically significant difference between Tulsi extract mouthwash and Placebo mouthwash group, Pomegranate peel extract mouthwash group and placebo mouthwash group, chlorhexidine mouthwash group and placebo mouthwash group. Between Tulsi extract mouthwash group, Pomegranate Peel extract mouthwash group and Chlorhexidine mouthwash group, no statistical significance was found. (Table 4)

TABLE 4: INTERGROUP COMPARISON OF MEAN *P. GINGIVALIS* COLONY FORMING UNITS AMONG GROUP I, GROUP II, GROUP III AND GROUP IV

Parameter	Visits	Group I (N=30) Mean ± SD	Group II (N=30) Mean ± SD	Group III (N=30) Mean ± SD	Group IV (N=30) Mean ± SD	P-value (ANOVA)	Post hoc (Bonferroni test)					
							Grp I vs Grp II	Grp II vs Grp III	Grp III vs Grp IV	Grp IV vs Grp I	Grp I vs Grp III	Grp II vs Grp IV
Patients Index	Visit1 (Baseline)	80800.00 ± 11121.27	82166.66 ± 17290.78	86566.66 ± 18222.16	87766.66 ± 14977.41	0.842	NS	NS	NS	NS	NS	NS
	Visit 2 (21 Days)	15266.66 ± 4282.54	16866.66 ± 3875.06	7633.33 ± 2399.47	30633.33 ± 6990.05	<0.001	NS	NS	<0.001	<0.001	NS	<0.001

Anova



Discussion

The present study assessed and compared the antiplaque and anti-gingivitis effect of Tulsi extract mouthwash and pomegranate peel extract mouthwash with Chlorhexidine mouthwash and placebo mouthwash. The results showed antiplaque and anti-gingivitis effect of Tulsi extract mouthwash and Pomegranate peel extract mouthwash were comparable to that of Chlorhexidine. The results demonstrated a significant reduction in the plaque index and gingival index scores after 21 days in all the groups.

A study by Gupta *et al.* [13] showed a significant reduction of plaque and gingival index scores when 4% tulsi mouthrinse was used over a period of 15 and 30 days. The results of the present study are in line with the study by Gupta *et al.*

The anti-gingivitis effect of Tulsi extract mouthwash can be attributed to compounds isolated from Tulsi extract. Civsilineol, Civsimavatine, Isothymonin, Apigenin, Rosavinic acid, and Eugenol compounds in tulsi were considered to have anti-inflammatory activity or cyclooxygenase inhibitory activity [14].

Similar results were obtained from a study by Somu *et al.*, showed excellent anti-gingivitis effect and significant reduction in plaque scores, using pomegranate gel (5g of carboxymethyl cellulose in 100mL of pomegranate juice) as an adjunct to mechanical debridement [15].

There was a significant reduction in the plaque score with pomegranate peel extract mouthwash compared to the group which used the placebo mouthwash. Possible explanation for this effect is the antibacterial agents present in pomegranate-hydrolysable tannins-that form complexes of a high molecular weight with soluble proteins, increase bacterial lysis, and moreover interfere with bacterial adherence mechanisms on tooth surfaces [16].



FIGURE 1: BEFORE SRP AND MOUTHWASH ADMINISTRATION, AT BASELINE



FIGURE 2: MEASUREMENT OF SULCUS DEPTH WITH WILLIAM'S PROBE, AT BASELINE



FIGURE 3: PER PROCEDURAL PLAQUE COLLECTION FROM BUCCAL SURFACE OF 16, USING GRACEY CURETTE. AT BASELINE



FIGURE 4: CULTURE POSITIVE *P. Gingivalis* COLONIES ON ENRICHED BLOOD AGAR, AT BASELINE



FIGURE 5: AFTER SRP AND MOUTHWASH ADMINISTRATION, AT 21 DAYS



FIGURE 6: PLAQUE COLLECTION FROM BUCCAL SURFACE OF 16, USING GRACEY CURETTE. AT 21 DAYS



FIGURE 7: CULTURE POSITIVE *P. Gingivalis* COLONIES ON ENRICHED BLOOD AGAR, AT 21 DAYS

CONCLUSION

In conclusion, this clinical study demonstrates that mouthwashes containing Tulsi extract and Pomegranate peel extract, when used in conjunction with scaling and root planing, exhibit significant anti-gingivitis and anti-plaque efficacy. Both interventions showed comparable effectiveness to Chlorhexidine, the gold standard of mouthwashes. These findings suggest that natural herbal extracts like Tulsi and Pomegranate could serve as viable alternatives to synthetic antimicrobial

agents in oral hygiene maintenance. Furthermore, future research could explore the long-term effects and potential side effects of these herbal mouthwashes, as well as investigate their efficacy in larger, diverse populations to establish their broader applicability in clinical practice.

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