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Comparing The Anti-Plaque And Anti-Gingivitis Efficacy Of Moringa Oleifera Mouthwash To Chlorhexidine And Saltwater Concentration In Treating Gingivitis: A Randomized Controlled Clinical Trial

Dr. Snigdha Kandukuri, Dr. Nagappa Guttiganur, Dr. Shivanand Aspalli, Dr.Bhavana

(IIIrd Year Postgraduate, Dept Of Periodontics, A.M.E's' Dental College, Raichur, Karnataka, India-584103)
(Professor, Dept Of Periodontics, A.M.E's' Dental College,Raichur,Karnataka,India-584103)
(Head Of The Department, Dept Of Periodontics, A.M.E's' Dental College, Raichur, Karnataka, India-584103)
(IIrd Year Postgraduate, Dept Of Periodontics, A.M.E's' Dental College, Raichur, Karnataka, India-584103)

Abstract:
Background: *Bacterial plaque is the primary cause of periodontal disease, necessitating its effective removal. Routine oral hygiene is essential, and chemical plaque control with agents like chlorhexidine can enhance treatment. However, side effects restrict its long-term use, prompting the search for alternative agents with fewer side effects. Ayurvedic treatments, such as Moringa oleifera, have been historically used for periodontal diseases. The nutrient-rich leaves of M. oleifera contain active compounds like flavonoids, alkaloids, phenolics, and triterpenoids, which offer antibacterial properties, making it a cost-effective antimicrobial agent.*
Objective: *This study aims to compare the antiplaque and anti-gingivitis efficacy of Moringa oleifera mouthwash to chlorhexidine and saltwater in gingivitis.*
Methodology: *The study involves three randomly selected groups (A, B, C), each consisting of 12 participants. All are advised to use 10ml of mouthwash for 1 minute, twice daily, 45 minutes after toothbrushing for 2 weeks, measured with 10ml cups. Questionnaire on discomfort (burning, itching, dryness, taste disturbance) and tooth discoloration*
Results: *The results have shown comparable effects of moringa oleifera and chlorhexidine mouthwash in plaque inhibition and reducing gingivitis. However, statistically significant differences have been seen when comparing M.oleifera and chlorhexidine with salt water concentration.*
Conclusion*: The Moringa Oleifera mouthwash exhibits significant plaque inhibition compared to salt water concentration and comparable effectiveness to chlorhexidine. Moreover, both the herbal and chlorhexidine mouthwashes show substantial reductions in gingivitis, indicating the potential of Moringa oleifera mouthwash as an alternative with similar efficacy.*
Key Word: *Moringa Oleifera, Mouthwash, Chlorhexidine, saline.*

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I. Introduction

Dental plaque, a tenacious yellowish-gray deposit, firmly adheres to intraoral hard surfaces and plays a crucial role in developing dental caries and gingival inflammation.[1] Microorganisms in the oral cavity form organized communities within a polysaccharide matrix known as dental biofilm, which can be effectively managed through proper oral hygiene practices. Gingivitis, marked by gingival inflammation, can affect individuals of all ages. Although brushing remains the primary self-care method for mechanically removing bacterial plaque, its effectiveness is often diminished by improper brushing techniques or plaque-retentive factors that encourage accumulation. As a result, chemical plaque control has emerged as a valuable and effective alternative for dental patients of all ages and conditions.[2].

The incorporation of chemical agents with antiplaque or antimicrobial properties into dental products has been proposed as a preventive strategy for reducing plaque-related conditions. Various antiseptic and antimicrobial agents have been utilized, demonstrating differing levels of success in inhibiting supragingival plaque formation and the progression of gingivitis.[3] Chlorhexidine (CHX), a broad-spectrum antimicrobial, is considered the gold standard for chemical plaque control. However, prolonged use of CHX mouthwash can lead to undesirable effects such as tooth and tongue discoloration, altered taste perception, and increased calculus formation, making it less suitable for long-term use.[4]

As a result, ongoing research is exploring alternative solutions, with a particular focus on biogenic agents as potential substitutes. Herbal dentifrices, classified as ethnomedicines, have gained popularity, though their efficacy varies based on phytopharmacological analysis.[5] Among these alternatives, *Moringa oleifera*, a widely cultivated tropical plant, particularly abundant in the sub-Himalayan regions, has shown promising potential. Renowned for its nutrient-rich leaves, which contain essential minerals, proteins, and vitamins, *Moringa oleifera* also boasts approximately 46 antioxidants that help neutralize free radicals in the body. Its active compounds, including flavonoids, tannins, saponins, alkaloids, phenolics, and triterpenoids, exhibit antibacterial properties, making it a compelling candidate for promoting oral health.[6]

The present study aims to compare the efficacy of the antiplaque & antigingivitis effect of Moringa oleifera mouthwash in comparison to chlorhexidine and saltwater concentration in gingivitis.

II. Material And Methods

The present study is a double-blinded, parallel designed randomized controlled trial carried out in the Dept. of Periodontics and Oral Implantology at A.M.E's Dental College and Hospital, Raichur, Karnataka. A total of 36 undergraduates (17 Males and 19 Females in the age group of 18-25 years) were included in this study. The ethical clearance was obtained from the ethical review committee of the institution. A written informed consent was obtained from the study participants after explaining in detail about the study.

Study Design: Double-blinded, designed randomized controlled trial.

Study Location: A.M.E's Dental college and hospital, Raichur, Karnataka

Study Duration: February 2024

Sample size: 36

Subjects & selection method: The study population included undergraduates studying in A.M.E's Dental College and Hospital, Raichur, Karnataka. The study comprises three groups (A, B, C), each with 12 participants chosen randomly through a simple lottery method as follows:
Group A (N=12 patients) - Moringa oleifera mouthwash
Group B (N=12 patients) – Chlorhexidine mouthwash
Group C (N=12 patients) **–** Salt water concentration

Inclusion criteria:
After taking a detailed medical history and initial clinical Examination,
1. Systemically healthy individuals with previously untreated gingivitis
2. The individuals with ≥ 20 teeth were selected.

Clinical parameters for inclusion are :
1. **OHI-S Index** (Greene and Vermillion 1964) – 0.1 - 3.0 Score
2. **Plaque Index** (Turesky et al Modification of the Quigley Hein (TQHPI) 1962) – ≤ 4 Score
3. **Gingival Index** (Loe and silness 1963) – 0.1-2.0 Score
4. **Probing depth** - ≤ 3mm

Exclusion criteria:

1) Use of Antibiotics & Anti-inflammatory drugs in the previous 6 months.
2) Individuals with orthodontic appliances or prosthetic appliances that would interfere with evaluation
3) Allergy to any ingredients used in the study.
4) Alcoholism.
5) Smokers or users of tobacco in any form.
6) pregnancy and Lactating females.

Study Design:

Group-A-Moringa Oleifera Mouthwash

Aqueous extract preparation:

Moringa oleifera leaves were harvested from the tree, subsequently washed with distilled water, dried in the shade, finely powdered **(Fig. A)**, and then preserved in a tight container.50gms of leaf powder is introduced into ethanol for aqueous extract preparation, the resulting mixture is subjected to soxhalet apparatus **(Fig. B)** for a total of 25 cycles. Subsequently, 250 ml of aqueous extract is obtained and subjected to vapourization until it reaches a volume of 250 ml.

Preparation of moringa oleifera mouthwash

To prepare 500ml of moringa oleifera mouthwash-In the process, 20ml of distilled water is combined with 250gms of Aspartame (a sweetening agent) **(Fig. C)**, and the resultant mixture is subjected to ultrasonication for 2 minutes to facilitate solvent dissolution **(Fig. D)**. Subsequently,0.2% (2ml) of an aqueous extract, 20ml of Glycerine (acting as a humectant for moisture retention) **(Fig. E)**, and 4 drops of peppermint oil (serving as a flavoring agent) **(Fig. F)** are incorporated into this solution. The final solution is then meticulously filtered through filter paper to ensure the removal of any residual particulate matter **(Fig. G)**. A total volume of 500ml mouthwash is diluted by combining it with an equal volume of distilled water resulting in preparation of 1 litre **(Fig. H).** The process is repeated in 3 batches ultimately yielding a total of 3 litres of mouthwash.

Group-B– Chlorhexidine mouthwash:

The present study uses a commercially available Chlorhexidine mouthwash (ChlorHex) containing 0.2% w/v.

Group-c – Saltwater concentration:

3 Litres of Distilled water are initially taken subsequently 500 ml of distilled water is separated and to this portion 7.5 gms of salt is added. The resulting solution is then diluted by combining it with 2500ml of additional distilled water.

A **B** **C** **D**

E **F** **G** **H**

Preparation of Moringa Oleifera Mouthwash

Study design:

An impartial individual was responsible for conducting the allocation and product coding in this study, which involved three groups (A, B, C) with 12 participants each, selected randomly through a simple lottery method. All 36 participants were instructed to use a soft toothbrush with toothpaste and practice a sulcular brushing technique for optimal oral care. The study followed a double-blind design, ensuring that both examiners and participants were unaware of the mouth rinses assigned. Participants used 10ml of mouthwash for 1 minute, twice daily after brushing their teeth for 2 weeks, with the quantity measured using 10ml cups.

Efficacy was assessed using:

- OHI-S index (Greene and Vermillion, 1964)
- Gingival index (Loe and Silness, 1963)
- Turesky-Gilmore-Glickman 1970 modification of the Quigley-Hein plaque index.

Assessments occurred at the study's baseline and after 2 weeks. For plaque inspection, a two-tone dye-disclosing agent covered all tooth surfaces. Gingivitis severity was evaluated across four scoring units: disto-facial papilla, facial margin, mesial facial papilla, and the entire lingual gingival margin. Additionally, participants completed a questionnaire regarding any discomfort (burning, itching, dryness, taste disturbance) and tooth discoloration.

Statistical analysis:

The data were systematically compiled, coded, computerized, and analyzed using SPSS version 26.0. One-way ANOVA and Paired t-test compared OHI-S, plaque, and gingival index values among three mouthwashes.

III. Results

Descriptives of OHI-S:

The table presents descriptive statistics for Oral Hygiene Index-Simplified (OHI-S) scores under different conditions, focusing on Moringa Oliefera Mouthwash, Chlorhexidine Mouthwash, and Saltwater Concentration at baseline and after 14 days. The OHI-S is a measure of oral hygiene, with higher scores indicating poorer oral hygiene.

Descriptives of OHI-S

						95% Confidence Interval for Mean	
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound
OHI Baseline	Moringa Oliefera Mouthwash	12	1.3583	.56320	.16258	1.0005	1.7162
	Chlorhexidine Mouthwash	12	.4250	.46147	.13321	1.1318	1.7182
	Saltwater Concentration	12	1.3333	.52281	.15092	1.0012	1.6655
	Total	36	1.3722	.50405	.08401	1.2017	1.5428
OHI 14 Days	Moringa Oliefera Mouthwash	12	11.0083	.49992	.14432	.6907	1.3260
	Chlorhexidine Mouthwash	12	1.0917	.40555	.11707	.8340	1.3493
	Saltwater Concentration	12	1.4833	.46872	.13531	1.1855	1.7811
	Total	36	1.1944	.49335	.08223	1.0275	1.3614

The mean OHI-S scores at baseline were relatively similar across the three groups, with Moringa Oleifera Mouthwash having a mean of 1.3583, Chlorhexidine Mouthwash at 1.4250, and Saltwater Concentration at 1.3333. The overall mean for all groups was 1.3722. Standard deviations indicate variability within each group, with Moringa Oleifera showing the highest variability. After 14 days, the mean OHI-S scores were 1.0083 for Moringa Oleifera, 1.0917 for Chlorhexidine, and 1.4833 for Saltwater Concentration, with the total mean for all groups decreasing to 1.1944. There was a noticeable reduction in mean OHI-S scores for the Moringa Oleifera and Chlorhexidine Mouthwash groups, indicating improved oral hygiene. In contrast, the Saltwater Concentration group showed a smaller reduction. The data indicate that both Moringa Oleifera and Chlorhexidine Mouthwash were effective in reducing OHI-S scores over 14 days, with Moringa Oleifera showing the most significant improvement, while Saltwater Concentration showed the least improvement among the three groups.

Descriptives of Gingival Index:

The table presents descriptive statistics for Gingival Index scores under different conditions, specifically focusing on Moringa Oliefera Mouthwash, Chlorhexidine Mouthwash, and Saltwater Concentration at baseline and after 14 days. The Gingival Index measures the severity of gingival inflammation, with higher scores indicating more severe inflammation.

Descriptives of Gingival Index							
						95% Confidence Interval for Mean	
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound
GingivalBaseline	Moringa Oliefera Mouthwash	12	.8833	.39274	.11337	.6338	1.1329
	Chlorhexidine Mouthwash	12	1.0167	.24802	.07160	.8591	1.1743
	Saltwater Concentration	12	.8583	.54349	.15689	.5130	1.2036
	Total	36	.9194	.40695	.06783	.7818	1.0571
Gingival14 Days	Moringa Oliefera Mouthwash	12	.3750	.31370	.09056	.1757	.5743
	Chlorhexidine Mouthwash	12	.2583	.18809	.05430	.1388	.3778
	Saltwater Concentration	12	.8583	.52822	.15248	.5227	1.1939
	Total	36	.4972	.44625	.07437	.3462	.6482

The baseline mean Gingival Index scores were similar across the groups, with Moringa Oleifera Mouthwash at 0.8833, Chlorhexidine Mouthwash at 1.0167, and Saltwater Concentration at 0.8583, and an overall mean of 0.9194. After 14 days, the mean scores were 0.3750 for Moringa Oleifera, 0.2583 for Chlorhexidine, and 0.8583 for Saltwater, with a total mean of 0.4972. Both Moringa Oleifera and Chlorhexidine Mouthwash groups showed significant reductions in scores, indicating improved gingival health, while the Saltwater Concentration group showed little improvement. The variability within each group remained similar except for the Saltwater group, which maintained higher variability. The data suggest Moringa Oleifera and Chlorhexidine Mouthwash were most effective in improving gingival health.

Descriptives of the Plaque Index

The table provides descriptive statistics for the Plaque Index scores under different conditions, specifically focusing on Moringa Oliefera Mouthwash, Chlorhexidine Mouthwash, and Saltwater Concentration at baseline and after 14 days.

Descriptives of Plaque Index							
						95% Confidence Interval for Mean	
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound
Plaque Baseline	Moringa Oliefera Mouthwash	12	1.8333	.71774	.20719	1.3773	2.2894
	Chlorhexidine Mouthwash	12	1.8333	.83485	.24100	1.3029	2.3638
	Saltwater Concentration	12	1.8333	.83485	.24100	1.3029	2.3638
	Total	36	1.8333	.77460	.12910	1.5712	2.0954
Plaque 14days	Moringa Oliefera Mouthwash	12	.9833	.43866	.12663	.7046	1.2620
	Chlorhexidine Mouthwash	12	.9083	.62152	.17942	.5134	1.3032
	Saltwater Concentration	12	1.6250	.64403	.18592	1.2158	2.0342
	Total	36	1.1722	.64702	.10784	.9533	1.3911

The baseline mean Plaque Index scores were identical across all groups, each at 1.8333, with an overall mean of 1.8333. After 14 days, the mean scores decreased to 0.9833 for Moringa Oleifera, 0.9083 for Chlorhexidine, and 1.6250 for Saltwater, with an overall mean of 1.1722. Both Moringa Oleifera and Chlorhexidine Mouthwash groups showed significant reductions in plaque scores, indicating improved oral hygiene, while the Saltwater Concentration group showed a smaller reduction. The data suggest Moringa Oleifera and Chlorhexidine Mouthwash were more effective in reducing plaque than Saltwater Concentration.

Paired Samples Test

The table presents the results of paired samples t-tests conducted on three variables (OHI, Gingival, and Plaque) measured at Baseline and after 14 days. For each variable, the table includes the mean difference

between Baseline and 14 Days, along with measures of variability (standard deviation, standard error of the mean) and the 95% confidence interval for the mean difference.

The t-value and degrees of freedom (df) are reported for each comparison, along with the significance level (Sig. 2-tailed). All three variables (OHI, Gingival, and Plaque) show statistically significant differences between Baseline and 14 Days, with p-values less than 0.001. This indicates strong evidence to reject the null hypothesis of no difference. Therefore, the results suggest that the interventions or conditions under study had a significant impact on oral health indicators within the study period.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	OHI_Baseline - OHI_14 Days	.17778	.27580	.04597	.08446	.27109	3.868	35	<0.001
Pair 2	Gingival_Baseline - Gingival_14 Days	.42222	.37956	.06326	.29380	.55065	6.674	35	<0.001
Pair 3	Plaque_Baseline - Plaque_14days	.66111	.56024	.09337	.47155	.85067	7.080	35	<0.001

One Way – ANOVA

The table displays the results of One-Way Analysis of Variance (ANOVA) tests conducted on different oral health indices at baseline and after 14 days. For OHI_Baseline, Gingival_Baseline, and Plaque_Baseline, the between-groups and within-groups sum of squares, degrees of freedom, mean squares, F-ratio, and significance levels (Sig.) are provided. No statistically significant differences were observed for OHI_Baseline and Gingival_Baseline as their p-values are greater than the conventional significance level of 0.05. In contrast, OHI_14 Days, Gingival_14 Days, and Plaque_14 Days show significant differences between groups, indicating that there are variations in these indices after the 14 days. These findings contribute valuable insights into the effectiveness of different treatments or interventions, providing a basis for further investigation into the impact on oral health outcomes.

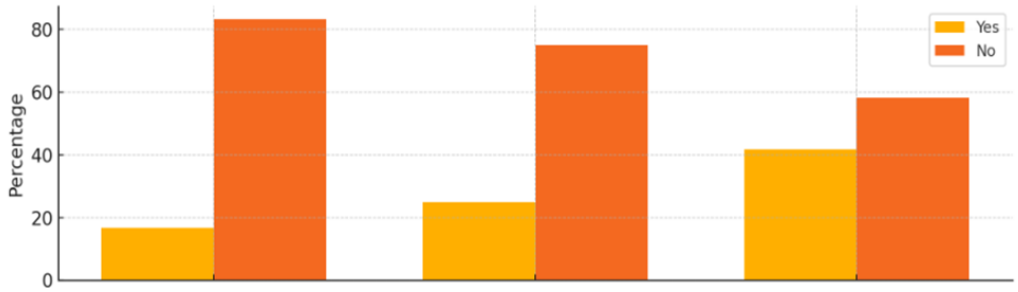
One Way - ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
OHI_Baseline	Between Groups	.054	2	.027	.101	.905
	Within Groups	8.838	33	.268		
	Total	8.892	35			
OHI_14 Days	Between Groups	1.544	2	.772	3.652	.037
	Within Groups	6.975	33	.211		
	Total	8.519	35			
Gingival_Baseline	Between Groups	.174	2	.087	.510	.605
	Within Groups	5.622	33	.170		
	Total	5.796	35			
Gingival_14 Days	Between Groups	2.429	2	1.214	8.826	.001
	Within Groups	4.541	33	.138		
	Total	6.970	35			
Plaque_Baseline	Between Groups	.000	2	.000	.000	1.000
	Within Groups	21.000	33	.636		
	Total	21.000	35			
Plaque_14days	Between Groups	3.724	2	1.862	5.622	.008
	Within Groups	10.928	33	.331		
	Total	14.652	35			

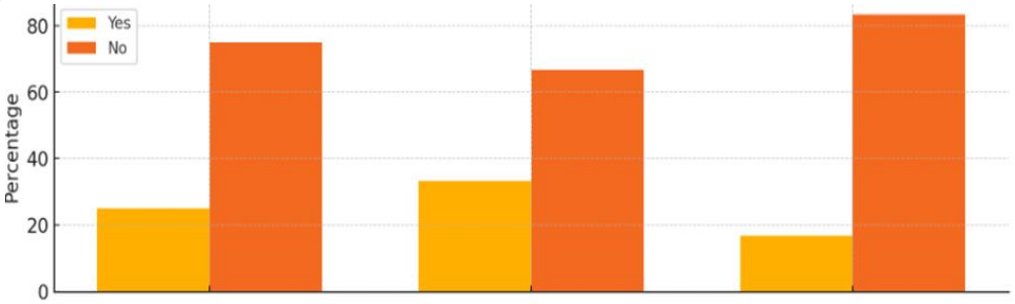
Descriptives of the questions

Here is the graph showing the percentages of "Yes" and "No" responses for each symptom across the three groups. Each subplot represents a different symptom, with the bars displaying the percentage of participants who experienced ("Yes") and did not experience ("No") each symptom in each group

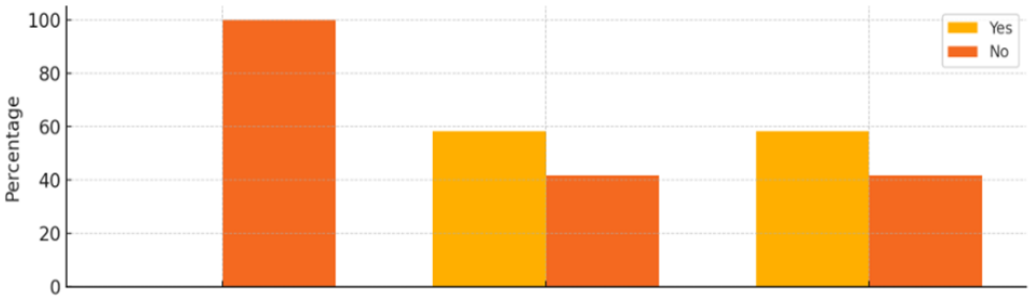
Discoloration Of Teeth



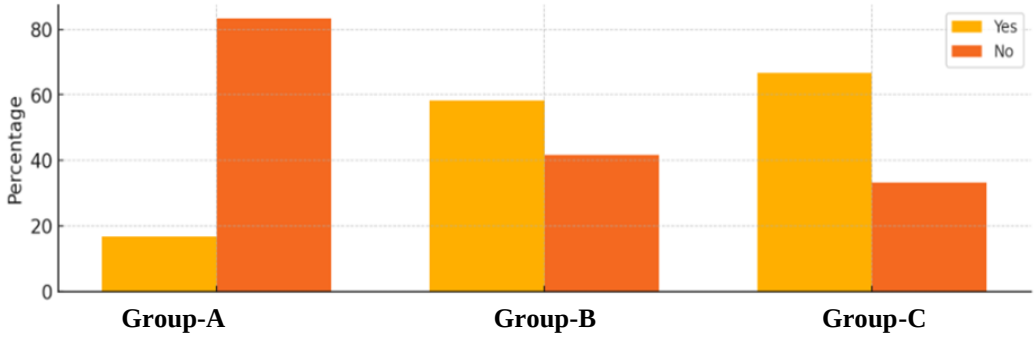
Burning Sensation



Dryness



Alteration In Taste



IV. Discussion

Plaque-induced gingivitis is a prevalent oral condition resulting from microbial biofilms on teeth. Inadequate oral hygiene can lead to severe gingival inflammation. While mechanical and chemical plaque control can help reverse inflammation, certain cases persist due to atypical infections caused by opportunistic microflora such as *Staphylococcus aureus, Enterococcus faecalis,* and *Candida albicans*.[7]

A literature review identified a limited number of studies comparing herbal mouthwashes to chlorhexidine mouthwash, with significant variations in sample size, study design, duration, assessment indices,

and techniques. Despite these differences, efforts were made to compare available findings while ensuring validity. Participants ranged in age from 18 to 24, with mean ages of 20.2±0.7 in the *Moringa oleifera* group, 20.95±1.1 in the chlorhexidine group, and 20.79±1.2 in the saline group. The absence of a significant difference ($p = 0.356$) among the groups indicates a homogeneous study population, aligning with findings from previous research.[8]

The similarity among groups indicates a homogeneous study population, consistent with previous research findings. Herbal medicines are increasingly utilized for treating various diseases, including periodontal conditions, due to their efficacy and fewer side effects compared to chemical treatments. As a result, Ayurvedic remedies are gaining popularity. Herbal oral rinses are currently used to manage gingival bleeding and inflammation.

Extensive research has explored the phytochemistry and pharmacological applications of *Moringa oleifera*. High-performance liquid chromatography of its ethanolic leaf extract has identified antioxidant polyphenols, including phenolic acids (ellagic and ferulic acid) and flavonoids (kaempferol, quercetin, vanillin, and rutin).[9]These polyphenols exhibit promising antimicrobial and immunomodulatory properties, making them potential agents for controlling periodontal diseases.[10]

Extensive studies have highlighted the therapeutic potential of *Moringa oleifera*. A systematic review analyzing its anti-inflammatory, antioxidant, and antiproliferative effects on cell lines identified kaempferol, quercetin, apigenin, and multiflora-B as key compounds responsible for its pharmacological benefits. The extracts selectively targeted cancer cells while remaining non-toxic to healthy cells. Among the various plant parts, the leaves demonstrated the most significant anti-inflammatory and antioxidant properties.[11]

Elagamily et al. conducted an in vitro study to assess the effectiveness of toothpaste and mouthwash formulated with *Moringa oleifera* (MO). They evaluated its antimicrobial properties against oral microbes, including *Staphylococcus aureus, Streptococcus mutans,* and *Candida albicans*. The toothpaste exhibited stronger antibacterial and antifungal activity compared to the mouthwash, which showed no antifungal effects. However, in the present study, the mouthwash demonstrated antibacterial activity.[12]

The antimicrobial activity of *Moringa oleifera* (MO) is attributed to its alkaloids, flavonoids, tannins, terpenoids, glycosides, and saponins. These phytochemicals enhance bacterial cell membrane permeability by inhibiting the enzyme sortase, disrupting DNA replication, and causing plasma membrane lysis. Tannins interfere with bacterial amino acid metabolism, slowing down bacterial reproduction, while saponins alter cell morphology and induce cell death by interacting with the cell membrane.[13] The ethanolic extract demonstrated the most potent antimicrobial activity against *Staphylococcus aureus* and *Streptococcus mutans* when compared to acetone and acetyl acetate extracts. Furthermore, the leaf extract exhibited the highest antimicrobial potential compared to other plant parts, such as seeds, roots, and mixtures. These findings are consistent with those of Rahman et al. (2013) and Devedra et al. (2011).[14,15]

Like the present study, Parwani et al.[16] and Sikka et al.[17] compared chlorhexidine and herbal mouthwash effectiveness using the Loe and Silness gingival index, as well as the Turesky et al. plaque index. Meanwhile, Scherer et al.[18] conducted a study comparing herbal mouthwash and distilled water, also employing the Loe and Silness gingival index. Additionally, Khalessi et al.[19] assessed the oral health benefits of Persica mouthwash compared to a placebo using the Silness and Loe plaque index.

Currently, this is the first in vivo study that has been conducted to evaluate the efficacy of Moringa Oleifera as a mouthwash for treating gingivitis. This study has shortcomings such as, including a short duration and a small sample size. It does not assess its impact on anaerobic organisms. Although the laboratory findings are promising, clinical trials are necessary to validate these results. Future research should consider cross-over trials with longer follow-up periods to extend the implications of these in vivo findings into clinical practice. Additionally, future studies should involve larger participant cohorts and include microbiological assessments for comprehensive evaluation.

V. Conclusion

The Moringa Oleifera mouthwash exhibits significant plaque inhibition compared to salt water concentration and have comparable effectiveness to chlorhexidine. Moreover, both the herbal and chlorhexidine mouthwashes show significant reductions in gingivitis, indicating the potential of Moringa oleifera mouthwash as an alternative with similar efficacy.

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