TPM Vol. 32, No. S7, 2025
ISSN: 1972-6325
https://www.tpmap.org/

Open Access

USE OF NEEM BARK AND KALONJI EXTRACT MOUTH WASH IN REDUCING PLAQUE INDEX; A CHALLENGE OR WAY FORWARD TO INCLUDE HERBAL COMPOUNDS IN COMMERCIAL USE

WAQAR-UN-NISA[1], ASMA ALI[2], TAYYABA MUMTAZ[3], SUGHRA ABID[4], RABIA INAM GANDAPORE[5], SAHAR KAMAL[6]

[1]ASSOCIATE PROFESSOR, DEPARTMENT OF ORAL PATHOLOGY, BACHA KHAN DENTAL COLLEGE, MARDAN, PAKISTAN
[2]ASSISTANT PROFESSOR, DEPARTMENT OF ORAL BIOLOGY, BACHA KHAN DENTAL COLLEGE, MARDAN, PAKISTAN
[3]ASSOCIATE PROFESSOR, JINNAH COLLEGE OF PHARMACY, DEPARTMENT OF PHARMACOGNOSY, SOHAIL UNIVERSITY, KARACHI, PAKISTAN
[4]LECTURER, DEPARTMENT OF DENTAL MATERIALS, KHYBER COLLEGE OF DENTISTRY, PESHAWAR, PAKISTAN
[5]ASSISTANT PROFESSOR, DEPARTMENT OF ANATOMY, BACHA KHAN DENTAL COLLEGE, MARDAN, PAKISTAN
[6]PHD SCHOLAR, CENTRE FOR PLANT CONSERVATION, KARACHI UNIVERSITY, KARACHI, PAKISTAN

CORRESPONDING AUTHOR: ASMA ALI
ASSISTANT PROFESSOR, DEPARTMENT OF ORAL BIOLOGY, BACHA KHAN DENTAL COLLEGE, MARDAN, PAKISTAN. EMAIL: KHAN_HOTI26@HOTMAIL.COM

ABSTRACT

Background: The antibacterial efficacy of neem bark and kalonji extract extends specifically to pathogenic bacteria implicated in common oral diseases. Among these, Streptococcus mutans plays a pivotal role in dental caries development due to its ability to adhere to tooth surfaces and produce acid that demineralizes enamel. Both neem and kalonji extracts have demonstrated significant inhibition of S. mutans growth and biofilm formation in vitro, thereby limiting cariogenic activity.

Objectives: To evaluate the antibacterial activity of both the extracts individually and in combination in comparison to commercially available mouth wash.

Methodology: It was a cross sectional study conducted in a tertiary care dental college of Karachi from June 2024 to August 2025. The powder of bark and kalonji was soaked into 70% ethanol with intermittent shaking for 5 days followed by rotary evaporation to get the extract. The extract was diluted in 10% dimethyl sulfoxide (DMSO) which was reduced to further dilution of 1% for each extract. The 120 students were selected randomly for the study after taking their verbal consent and they were divided into 4 groups (30 participants per group). All the students were instructed to not brush their teeth for a night and in the morning plaque index was checked after that they were allowed to brush their teeth. The procedure was repeated for 3 nights and plaque index was evaluated. All the students were instructed to use the provided mouth wash immediately after the dinner (last meal of the day) and for three consecutive days plaque index was measured again. The data was compared as pre and post (before using mouth wash and after using mouth wash) by paired t test and, among the groups by ANOVA followed by post hoc analysis.

Results: The ANOVA analysis of plaque index first three (before intervention) readings showed insignificant results, among all the participants. The pre and post intervention analysis among all the groups showed a significant (p-value < 0.05) decrease in plaque index which was comparable to commercially available mouth wash.

Conclusion: The neem bark, kalonji extract alone and in combination reduced plaque index comparable to the chlorhexidine.

Keywords: Neem bark, kalonji extract, mouth wash, plaque index; herbal compounds

INTRODUCTION

Neem bark and kalonji extract have garnered considerable attention in recent years for their potent antibacterial properties, particularly when explored as natural alternatives in oral care products. Neem (Azadirachta indica), traditionally revered in Ayurvedic medicine, is rich in bioactive compounds such as nimbin, nimbolide, and azadirachtin, which contribute to its wide spectrum of antimicrobial activity (Jihad et al., 2021; Mudenda, Banda,

Mohamed, Chabalenge, & Phytochemistry, 2023). Similarly, kalonji (Nigella sativa), commonly known as black seed, contains thymoquinone and other phytochemicals known for their bactericidal and anti-inflammatory effects. The combination of neem bark and kalonji extract offers a synergistic potential in inhibiting a broad range of bacteria, making them promising candidates for developing herbal mouthwashes aimed at reducing oral bacterial load and promoting oral hygiene (Faizah, Suparno, Faza, & Sciences, 2024).

The antibacterial efficacy of neem bark and kalonji extract extends specifically to pathogenic bacteria implicated in common oral diseases . Among these, Streptococcus mutans plays a pivotal role in dental caries development due to its ability to adhere to tooth surfaces and produce acid that demineralizes enamel (Chaturvedi). Both neem and kalonji extracts have demonstrated significant inhibition of S. mutans growth and biofilm formation in vitro, thereby limiting cariogenic activity (Joel David, 2021). Furthermore, these extracts show activity against other oral pathogens such as Porphyromonas gingivalis and Fusobacterium nucleatum, which are involved in periodontal diseases. Their antimicrobial action disrupts bacterial cell walls and impairs virulence factors, reducing plaque formation and gingival inflammation, which underscores their therapeutic potential (Kamali, Rezvani, Pourhajibagher, Farzaneh, & Emami-Razavi, 2024).

Given the increasing concerns about side effects associated with conventional medicinal mouthwashes including mucosal irritation, altered taste, and potential antimicrobial resistance there is a growing interest in herbal alternatives like neem and kalonji-based mouth rinses (Petrovski, Terzieva-Petrovska, Taskov, & Papakoca, 2022). These natural extracts typically exhibit fewer adverse effects, making them safer for long-term use. Furthermore, herbal mouthwashes integrate additional benefits such as antioxidant and anti-inflammatory properties, contributing to overall oral health beyond simple antimicrobial action (Anwar et al., 2025). This rationale supports ongoing research and development focused on harnessing neem and kalonji extracts as effective, well-tolerated mouthwash components, offering a natural and holistic approach to oral care. Considering, the reported effectiveness of neem bark and kalonji extracts this study was aimed to evaluate the antibacterial activity of both the extracts individually and in combination in comparison to commercially available mouth wash.

METHODOLOGY

It was a cross sectional study conducted in a tertiary care dental college of Karachi from June 2024 to august 2025. The neem tree bark and kalonji were purchased from local market of Karachi. The bark and kalonji seeds were washed and dried under the shade for 3 days after that they were grinded to powder. The powder of bark and kalonji was soaked into 70% ethanol with intermittent shaking for 5 days followed by rotary evaporation to get the extract. The extract was diluted in 10% dimethyl sulfoxide (DMSO) which was reduced to further dilution of 1% for each extract. The 120 students were selected randomly for the study after taking their verbal consent and they were divided into 4 groups (30 participants per group). All the students were instructed to not brush their teeth for a night and in the morning plaque index was checked after that they were allowed to brush their teeth. The procedure was repeated for 3 nights and plaque index was evaluated. The plaque index was measured as 0 for no plaque, 1 for thin film of plaque at gingival margin, 2 for visible moderate plaque and 3 for heavy/abundant plaque (Carvalho, Moura, Costa, Cota, & Dentistry, 2023). Following the plaque index measurement, the groups were assigned the experimental drugs, group 1 was assigned only neem tree bark extract, group 2 was assigned only kalonji extract, group 3 was assigned combination of neem tree bark and kalonji extract and group 4 was assigned commercially available mouth wash (chlorhexidine). All the students were instructed to use the provided mouth wash immediately after the dinner (last meal of the day) and for three consecutive days plaque index was measured. According to plaque index results were generated in mean and SD. The data was compared as pre and post (before using mouth wash and after using mouth wash) by paired t test and, among the groups by ANOVA followed by post hoc analysis. SPSS version 27 was used for the data analysis and p value less than 0.05 was considered as significant.

RESULTS

There were 120 (100%) students, who were enrolled after the consent in the study. Among them, 79 (65.83%) were females and 41 (34.17%) were female. The mean age of participants was 21.2 ± 2.1. The ANOVA analysis of plaque index first three (before intervention) readings showed insignificant results, among all the participants. The plaque index reading was showing visible moderate plaque identified as 2. The over all mean was 2.34 ± 0.17 however, group wise mean is described in table 1.

Table 1. The plaque index of participants (group wise) before intervention.

Groups	**Plaque index (before intervention) (Mean ± SD)**	**(p- value)**
1	2.1 ± 0.51	1.000

2	2.34 ± 0.36	
3	2.7 ± 0.11	
4	2. 22 ± 0.33	

As per grouping the intervention was performed for three consecutive nights. The students were instructed to use the provided mouthwash after the dinner and in the morning their plaque index was evaluated. The pre and post intervention analysis among all the groups showed a significant (p-value < 0.05) decrease in plaque index which was comparable to commercially available mouth wash, table 2 shows the pre and post interventional values of plaque index among the groups. There was no significant difference between the group which showed that if neem tree bark or kalonji extract is used individually or in combination will show same effects in decreasing the plaque index which is comparable to chlorhexidine (table 3).

Table 2. Pre and post interventional values of plaque index among the groups

Groups	**Plaque index (Mean ± SD)**		**(p- value)**
	Before intervention	**After intervention**	
1	2.1 ± 0.51	1.33 ± 0.12	0.031
2	2.34 ± 0.36	1.28 ± 0.33 0	0.012
3	2.7 ± 0.11	1.01 ± 0.10	0.001
4	2. 22 ± 0.33	1.18 ± 0.16	0.044

Table 3. The plaque index of participants (group wise) after intervention.

Groups	**Plaque index (After intervention) (Mean ± SD)**	**(p- value)**
1	1.33 ± 0.12	1.000
2	1.28 ± 0.33 0	
3	1.01 ± 0.10	
4	1.18 ± 0.16	

DISCUSSION

Neem bark and kalonji extract have received widespread attention for their exceptional potential as natural constituents in mouthwash formulations, combining centuries of traditional wisdom with current scientific proof. Neem bark, produced from the Azadirachta indica tree, is well-known for its robust antibacterial, anti-inflammatory, and antifungal qualities, making its bark extract an effective oral hygiene treatment (Jihad et al., 2021; Mudenda et al., 2023). Neem bark has chemicals that successfully attack oral microorganisms that cause dental plaque, gum disease, and foul breath, while also maintaining healthy gums and lowering inflammation (Khan et al., 2022). Kalonji, or Nigella sativa, has comparable advantages because to its high concentration of bioactive substances such thymoquinone, which has significant antioxidant, antibacterial, and anti-inflammatory properties. Its extract promotes dental health by limiting the growth of dangerous bacteria and fungus, which aids in the prevention (Bhavikatti et al., 2024; Wylie & Merrell, 2022). With rising consumer demand for plant-based, chemical-free products, mouthwashes containing these extracts have the potential to be long-term, safe alternatives to conventional products, providing benefits like cavity prevention, gum strengthening, and anti-inflammatory effects with few side effects. Furthermore, their antioxidant properties help to neutralize free radicals in the oral cavity, potentially lowering the risks associated with oxidative stress and chronic oral diseases. Emerging clinical trials reveal encouraging findings supporting these advantages, but further research is needed to optimize their concentration and formulation stability for the best therapeutic effects (Anwar et al., 2025; Bhavikatti et al., 2024; Kumar, 2024; Singh et al., 2021).

This research investigated the efficiency of several mouthwash formulations in decreasing dental plaque. Participants were divided into four groups, each getting a different treatment for three nights. Group 1 used a mouthwash containing only neem tree bark extract, Group 2 used only kalonji extract, Group 3 received a combination of neem bark and kalonji extracts, and Group 4 used a commercially available mouthwash containing chlorhexidine, which is widely recognized for its strong antimicrobial properties and is considered a gold standard in plaque control.

The intervention procedure required students to rinse with their designated mouthwash after supper on three consecutive nights. On the mornings after the intervention, each participant's plaque index—an established clinical measure of dental plaque accumulation—was assessed. The study found that all treatments groups had a significant decrease in plaque index compared to their baseline values (p-value < 0.05). This suggests that all types of mouthwash, including natural extract-based formulations, were successful in decreasing plaque throughout the short trial period which is similar to documented data (Lee et al., 2021; Tzimas, Antoniadou, Varzakas, & Voidarou, 2024). More significantly, the level of plaque reduction found in the neem bark extract, kalonji extract, and combination groups was equivalent to that achieved by a commercial mouthwash containing chlorhexidine. This discovery highlights the potential of neem bark and kalonji extracts, both individually and synergistically, as natural alternatives to typical chemical mouthwashes. Neem bark's antimicrobial and anti-inflammatory constituents are likely to have helped inhibit plaque-forming bacteria and improve gingival health, while kalonji's bioactive compounds, such as thymoquinone, may have amplified these effects by providing additional antioxidant and antimicrobial activity (Aneja, Mittal, Dhiman, & Agarwal, 2023; Arora, Sen, & strategies, 2023; Shahzadi, Yousaf, Aftab, Riaz, & Wahab, 2023). The combination mouthwash may have exploited complementary methods of action, increasing total effectiveness (Naeem, Iqbal, Roohi, & Sciences, 2022). Given chlorhexidine's recognized negative effects, such as tooth discoloration and changed taste, the equivalent efficiency of these natural extracts makes them an appealing, safer alternative for everyday dental care (Deus & Ouanounou, 2022). The brief intervention time resulted in meaningful effects, indicating that these agents operate quickly to prevent plaque development. However, longer-term trials might be beneficial in determining long-term effectiveness and safety.

Overall, this study emphasizes the prospective significance of neem bark and kalonji extract-based mouthwashes in oral health management, suggesting that natural plant extracts can be efficient and well-tolerated alternatives to traditional chemical mouthwashes such as chlorhexidine. Their ability to drastically lower plaque index within three days lays the path for more study and possible commercial development of herbal oral care products that match rising customer demand for natural, side-effect-free choices. The study's limitations include its short length of only three consecutive nights, which may not accurately reflect the mouthwashes' long-term effectiveness or negative effects. The sample size and demographic variety of participants were not reported, which may restrict generalizability. Furthermore, relying simply on the plaque index without considering other oral health variables such as gingival inflammation or microbiota alterations limits outcome interpretation. Future study should include bigger, more varied groups and a longer intervention period to assess long-term effectiveness and safety. Comprehensive oral health examinations and research into ideal extract concentrations will help establish neem bark and kalonji as natural mouthwash alternatives.

CONCLUSION

The neem bark, kalonji extract alone and in combination reduced plaque index comparable to the chlorhexidine. There were no synergistic effects of neem bark an kalonji extract in reducing plaque index when used in combination.

REFERENCES

1. Aneja, S., Mittal, A., Dhiman, N., & Agarwal, G. J. P. R. (2023). A Review on Phytoconstituents for the Treatment of Psoriasis. *17*(34).
2. Anwar, M. A., Sayed, G. A., Hal, D. M., Hafeez, M. S. A. E., Shatat, A.-A. S., Salman, A., . . . Hatem, S. J. I. (2025). Herbal remedies for oral and dental health: a comprehensive review of their multifaceted mechanisms including antimicrobial, anti-inflammatory, and antioxidant pathways. *33*(3), 1085-1160.
3. Arora, K., Sen, S. J. P. f. i., & strategies, c. (2023). Medicinally important phytoconstituents and conservation strategies of neem: a critical overview. 21-44.
4. Bhavikatti, S. K., Zainuddin, S. L. A., Ramli, R. B., Nadaf, S. J., Dandge, P. B., Khalate, M., & Karobari, M. I. J. S. R. (2024). Insights into the antioxidant, anti-inflammatory and anti-microbial potential of Nigella sativa essential oil against oral pathogens. *14*(1), 11878.
5. Carvalho, A.-P., Moura, M.-F., Costa, F.-O., Cota, L.-O.-M. J. J. o. C., & Dentistry, E. (2023). Correlations between different plaque indexes and bleeding on probing: A concurrent validity study. *15*(1), e9.
6. Chaturvedi, A. Page Title: Efficacy of a Self–Prepared Azadirachta indica Mouthwash on Porphyromonas gingivalis Colonies: Invitro Microbiological Study.
7. Deus, F. P., & Ouanounou, A. J. I. d. j. (2022). Chlorhexidine in dentistry: pharmacology, uses, and adverse effects. *72*(3), 269-277.
8. Faizah, A., Suparno, N. R., Faza, M. A. J. J. M., & Sciences, C. (2024). The influence of mouthwash with habbatussauda extract (Nigella sativa) in inhibiting the growth of Streptococcus mutans bacteria (in vitro). *7*(8), 1033-1042.

9. Jihad, M. A., Noori, F. T., Jabir, M. S., Albukhaty, S., AlMalki, F. A., & Alyamani, A. A. J. M. (2021). Polyethylene glycol functionalized graphene oxide nanoparticles loaded with nigella sativa extract: a smart antibacterial therapeutic drug delivery system. *26*(11), 3067.
10. Joel David, S. (2021). *Effectiveness Of Azardirachta Indica (Neem Extract) Mouth Rinse On Plaque And Gingival Health-An Interventional Study.* Bbdcods,
11. Kamali, S.-A., Rezvani, M.-B., Pourhajibagher, M., Farzaneh, F., & Emami-Razavi, H.-S. J. G. M. J. (2024). In Vitro Synergistic Antibacterial Effects of Extract and Honey Derived from Nigella Sativa on Streptococcus Mutans: Antibacterial Effects of Extract and Honey Derived from Nigella Sativa on Streptococcus Mutans. *13*(SP1), e3567-e3567.
12. Khan, S. D., Ramzan, A., Alyami, M. N., Dhaen, A. M., Almakrami, A. H., Almakrami, A. A., . . . Alyami, M. M. J. S. J. M. P. S. (2022). Pharmacological Aspects of Traditional Plant Extracts in Maintaining Oral Health: A Narrative Review. *8*(11), 653-659.
13. Kumar, M. J. E. C. J. (2024). Antibacterial and anti-inflammatory potential of Azadirachta indica against dental bacteria. *25*(4), 1140-1149.
14. Lee, S.-H., Kim, W.-H., Ju, K.-W., Lee, M.-S., Kim, H.-S., Lee, J.-H., . . . Kim, B.-J. J. A. S. (2021). Antibacterial and anti-inflammatory potential of mouthwash composition based on natural extracts. *11*(9), 4227.
15. Mudenda, S., Banda, M., Mohamed, S., Chabalenge, B. J. J. o. P., & Phytochemistry. (2023). Phytochemical composition and antibacterial activities of azadirachta indica (Neem): significance of traditional medicine in combating infectious diseases and antimicrobial resistance. *12*(5), 256-263.
16. Naeem, M., Iqbal, Z., Roohi, N. J. J. J. o. A., & Sciences, P. (2022). Efficacy of neem (Azadirachta indica), Shahtra (Fumaria parviflora) leaves and Kalonji (Nigella sativa) seeds against Haemonchus contortus infection in locally bred Rambouillet sheep in Pakistan. *32*(5).
17. Petrovski, M., Terzieva-Petrovska, O., Taskov, T., & Papakoca, K. J. M. p. b. (2022). Side effects associated with chlorhexidine mouthwashes use. *68*(sup 1), 377-378.
18. Shahzadi, Z., Yousaf, Z., Aftab, A., Riaz, M., & Wahab, S. (2023). Kalonji. In *Essentials of Medicinal and Aromatic Crops* (pp. 735-762): Springer.
19. Singh, V., Roy, M., Garg, N., Kumar, A., Arora, S., & Malik, D. S. J. R. A. i. A.-I. D. D. F. R. P. o. A.-I. D. D. (2021). An insight into the dermatological applications of neem: a review on traditional and modern aspect. *16*(2), 94-121.
20. Tzimas, K., Antoniadou, M., Varzakas, T., & Voidarou, C. J. C. I. i. M. B. (2024). Plant-derived compounds: A promising tool for dental caries prevention. *46*(6), 5257-5290.
21. Wylie, M. R., & Merrell, D. S. J. F. i. p. (2022). The antimicrobial potential of the neem tree Azadirachta indica. *13*, 891535.