

Formulation and Evaluation of Polyherbal Mouthwash Using Neem And Tulsi

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Abstract: *The present study was carried out to develop and preliminarily evaluate a polyherbal mouthwash containing Neem and Tulsi extracts in combination with clove and peppermint oils. The formulation comprised Neem extract, Tulsi extract, clove oil, peppermint oil, glycerin, sodium benzoate and purified water. The herbal extracts were prepared separately and incorporated into the formulation followed by addition of the remaining ingredients, mixing, filtration and storage. The prepared formulation was assessed for organoleptic characteristics, pH, homogeneity, clarity, shake test, antimicrobial activity, irritancy, viscosity, stability and preliminary phytochemical constituents. The formulation showed a yellowish-green colour, pleasant aromatic odour, slightly bitter and refreshing taste, smooth appearance and clear nature. The measured pH was 6.4, and the formulation remained homogeneous without visible precipitation or phase separation after shaking. Antimicrobial activity was observed against the tested oral microorganisms, while no observable irritation was reported under the stated test conditions. Qualitative phytochemical screening indicated alkaloids, flavonoids, tannins, phenolic compounds, glycosides and terpenoids. These findings provide preliminary evidence of acceptable physical characteristics and support further investigation of the formulation.*

Keywords: Polyherbal mouthwash; Neem; Tulsi; Oral hygiene; Antimicrobial activity; Herbal formulation; Phytochemical screening.

I. INTRODUCTION

Maintaining oral hygiene is essential for preserving the health of the teeth, gingiva and other oral tissues. Inadequate oral-care practices may favour plaque accumulation and are associated with conditions including dental caries, gingivitis, periodontitis, halitosis and other oral infections. Regular oral-care practices, including tooth brushing, flossing, tongue cleaning, and the use of mouthwash, are commonly employed to maintain oral cleanliness and support oral health ^[1,2].

Mouthwash is an oral liquid preparation generally used in addition to routine measures such as brushing and flossing. Depending on its composition, it may assist in oral cleansing, breath freshening and control of microorganisms within the oral cavity. Mouthwash can also help reach areas of the oral cavity that may not be adequately accessed through routine brushing. Conventional mouthwash formulations may contain synthetic antimicrobial agents, preservatives, flavouring substances, alcohol, and other excipients. Certain formulations may be associated with undesirable effects such as tooth staining, burning sensation, dry mouth, altered taste sensation, or oral irritation, depending on their composition and use ^[2,3]. Interest in plant-based oral-care preparations has increased because medicinal plants provide diverse phytochemical constituents that may contribute to biological activities relevant to oral health. Reported constituents include alkaloids, flavonoids, tannins, phenolic compounds, glycosides, terpenoids and essential oils, which have been associated with antimicrobial, anti-inflammatory, antioxidant and antiseptic activities. Consequently, herbal and polyherbal formulations have been investigated for their potential applications in oral healthcare ^[4,5].

Neem (*Azadirachta indica*) is a medicinal plant traditionally used in herbal medicine and has been reported to possess antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, and antiseptic properties. Neem has also been investigated for applications in oral care, including its potential role in controlling microbial growth and supporting



dental and gingival health ^[14-16]. **Tulsi (*Ocimum sanctum*)**, commonly known as holy basil, is another medicinal plant widely used in traditional systems of medicine. It has been reported to possess antibacterial, anti-inflammatory, antioxidant, and other pharmacological properties, making it a potential herbal ingredient for oral-care formulations ^[19-21].



Fig no.1:- Neem



Fig no.2:- Tulsi

Clove oil obtained from *Syzygium aromaticum* is traditionally associated with dental and oral-care applications. It contains biologically active constituents and has been reported to possess antibacterial, antiseptic, analgesic, antioxidant, and anti-inflammatory properties ^[22-24]. **Peppermint oil** obtained from *Mentha piperita* is commonly used in oral-care preparations because of its characteristic flavour, cooling sensation, and breath-freshening properties. Its incorporation may also contribute to the organoleptic characteristics of a mouthwash formulation ^[26,27].

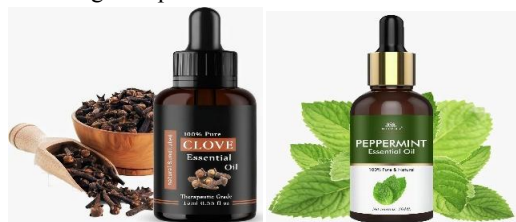


Fig no.3:- Clove Oil

Fig no.4:- Peppermint oil

Earlier investigations have explored mouthwash formulations incorporating combinations of medicinal plant extracts and essential oils, with emphasis on physical quality attributes and antimicrobial performance ^[12,13,25]. Such studies provide a useful background for examining additional herbal combinations for oral-care applications. Based on this background, the present study was undertaken to formulate and evaluate a polyherbal mouthwash containing Neem extract and Tulsi extract along with clove oil and peppermint oil. The prepared formulation was evaluated for organoleptic characteristics, pH, homogeneity, clarity, shake stability, antimicrobial activity, irritancy, viscosity, stability, and preliminary phytochemical constituents.

II. LITERATURE REVIEW

Sr.no.	Author's & Year	Herbal Formulation	Methodology	Key Findings
1	Jain et al. (2022) ^[13]	Polyherbal mouthwash containing guava leaves, turmeric, clove, peppermint, cinnamon and other ingredients	Formulation and evaluation including appearance, colour, odour, pH and antimicrobial assessment	The study supported the potential use of combinations of herbal ingredients in mouthwash formulations for oral-care applications.
2	Arthi et al. (2023) ^[25]	Herbal mouthwash containing Neem, white tea, cinnamon, basil and	Development and evaluation of the herbal mouthwash for	The study demonstrated the relevance of medicinal plants and essential oils in the development of herbal oral-care



		peppermint	antimicrobial and anti-inflammatory potential	formulations.
3	Mahajan et al. (2021) ^[12]	Polyherbal mouthwash containing <i>Piper betel</i> , Neem, Tulsi, clove, mint and other ingredients	Formulation and evaluation including antibacterial activity, pH, clarity and stability	The study reported antibacterial activity and evaluated important physicochemical characteristics of the polyherbal mouthwash.

Table 1: Summary of Previous Studies on Herbal and Polyherbal Mouthwash Formulations

III. AIM AND OBJECTIVES

Aim:

To formulate and evaluate a polyherbal mouthwash containing Neem and Tulsi extracts along with clove oil and peppermint oil.

Objectives:

1. To formulate a polyherbal mouthwash using Neem and Tulsi extracts with selected excipients and essential oils.
2. To evaluate the prepared formulation for organoleptic characteristics such as colour, odour, taste, appearance, and clarity.
3. To evaluate the physicochemical characteristics of the prepared mouthwash, including pH, homogeneity, and physical stability.
4. To evaluate the antimicrobial activity of the prepared polyherbal mouthwash against the tested oral microorganisms.
5. To assess the irritancy and viscosity of the prepared formulation.
6. To evaluate the stability of the prepared mouthwash during storage.
7. To perform preliminary phytochemical screening of the formulation for the presence of selected phytoconstituents.

IV. MATERIALS AND METHODS :

1. Ingredients:

Table 2: Composition of the Prepared Polyherbal Mouthwash Formulation

Sr.no.	Ingredients	Quantity Used	Role
1	Neem Extract	400 mg	Antibacterial and antiseptic agent
2	Tulsi Extract	400 mg	Antimicrobial and anti-inflammatory agent
3	Clove Oil	0.2 ml	Analgesic, antiseptic, and flavoring agent
4	Peppermint Oil	0.05 ml	Cooling and breath-freshening agent
5	Glycerin	5 ml	Sweetening and viscosity enhancing agent
6	Sodium Benzoate	100 mg	Preservative
7	Purified Water	q.s to 100 mL	Vehicle

2. Apparatus Required :

The apparatus used included beakers, measuring cylinders, glass rods, funnels, filter paper, droppers, an electronic weighing balance, stirrer and storage bottles.

Method of Preparation :

Step 1: Preparation of Neem Extract

- Weigh the required quantity of Neem powder accurately.

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- Mix the Neem powder with distilled water.
- Heat the mixture gently with continuous stirring.
- Filter the extract using filter paper.
- Collect the clear Neem extract.

Step 2: Preparation of Tulsi Extract

- Weigh the required quantity of Tulsi powder accurately.
- Mix the Tulsi powder with distilled water.
- Heat the mixture gently with continuous stirring.
- Filter the extract properly.
- Collect the clear Tulsi extract.

Step 3: Preparation of Base Solution

- Weigh the required quantity of Sodium benzoate accurately.
- Dissolve Sodium benzoate in a small quantity of purified water.
- Add Glycerin into the solution.
- Stir the mixture properly to obtain uniform solution.

Step 4: Addition of Herbal Extracts

- Add the prepared Neem extract slowly into the base solution.
- Add Tulsi extract gradually into the formulation.
- Stir continuously for proper mixing.

Step 5: Addition of Essential Oils

- Add measured quantity of Clove oil slowly into the formulation.
- Add Peppermint oil carefully into the mixture.
- Continue stirring for uniform distribution of oils.

Step 6: Addition of Vehicle

- Add purified water gradually into the formulation.
- Adjust the final volume up to 100 mL.

Step 7: Mixing of Formulation

- Stir the entire formulation continuously for sufficient time.
- Obtain homogeneous and uniform mouthwash preparation.

Step 8: Filtration

- Filter the prepared mouthwash using filter paper.
- Remove unwanted particles and impurities.

Step 9: Filling and Storage

- The final formulation was transferred into a clean and dry container.
- Store the formulation at room temperature for further evaluation studies.



V. EVALUATION PARAMETERS

1. Organoleptic Evaluation:

Procedure: • The prepared mouthwash was visually evaluated for colour, odour, taste, appearance, and clarity.

Purpose: • To determine the physical appearance and acceptability of the prepared mouthwash formulation.

2. pH Determination :

Procedure: • Take a suitable quantity of prepared mouthwash.

- Measure the pH using a pH Paper.

Purpose: • To determine the acidity or alkalinity of the formulation.

- To ensure compatibility of the formulation with the oral cavity.

3. Homogeneity Test :

Procedure: • Observe the prepared mouthwash visually against light.

- Check the formulation for uniformity and smooth appearance.

Purpose : • To determine uniform distribution of ingredients in the formulation.

- To ensure absence of lumps and aggregation.

4. Clarity Test :

Procedure : • Observe the mouthwash carefully against light and dark background.

- Check for clarity and presence of suspended particles.

Purpose : • To determine transparency and clarity of the formulation.

- To ensure absence of unwanted particulate matter.

5. Shake Test :

Procedure: • Mouthwash was shaken thoroughly and visually examined for changes in uniformity, precipitation, or phase separation.

Purpose : • To evaluate physical stability of the mouthwash.

- To observe uniformity of the formulation after shaking.

6. Antimicrobial Study :

Procedure: • Prepare culture media suitable for oral microorganisms.

- Inoculate the microorganisms on sterile agar plates.
- Prepare wells in the agar medium using sterile borer.
- Add the prepared polyherbal mouthwash into the wells.
- Incubate the plates at suitable temperature for 24 hours.
- Observe the zone of inhibition around the wells.

Purpose: • To evaluate antimicrobial activity of the prepared mouthwash against oral microorganisms.

- To determine effectiveness of herbal ingredients in controlling microbial growth.

7. Irritancy Test :

Procedure: • Apply a small quantity of prepared mouthwash on oral mucosal surface.

- Observe for redness, irritation, swelling, or discomfort after application.

Purpose: • To determine safety of the formulation.

- To evaluate non-irritant nature of the prepared mouthwash.



8. Viscosity Determination :

Procedure: • Take suitable quantity of mouthwash formulation.
• Measure viscosity using suitable viscometer at room temperature.

Purpose: • To determine flow property and consistency of the formulation.
• To ensure proper pouring and handling characteristics.

9. Stability Study :

Procedure: • Store the prepared mouthwash formulation at room temperature.
• Observe the formulation periodically for changes in: Colour, odor, appearance, pH, Clarity.

Purpose: • To evaluate physical stability of the formulation during storage.
• To determine suitability of formulation for longer storage period.

VI. PHYTOCHEMICAL SCREENING :

Table 3: Phytochemical Screening Tests Used for the Polyherbal Mouthwash

Sr.no.	Test	Procedure
1	<u>Test for Alkaloids:</u> Dragendorff's Test	Take 2 mL of polyherbal mouthwash in a test tube and add a few drops of Dragendorff's reagent.
2	<u>Test for Flavonoids:</u> Alkaline Reagent Test	Take 2 mL of mouthwash and add a few drops of 10% sodium hydroxide solution.
3	Test for Tannins: Ferric Chloride Test	Take 2 mL of mouthwash and add 2–3 drops of 5% ferric chloride solution.
4	<u>Test for Phenolic Compounds:</u> Ferric Chloride Test	Take 2 mL of mouthwash and add a few drops of ferric chloride solution.
5	<u>Test for Glycosides:</u> Keller-Killiani Test	Take 2 mL of mouthwash, add glacial acetic acid containing ferric chloride, then carefully add concentrated sulfuric acid along the side of the test tube.
6	<u>Test for Terpenoids:</u> Salkowski Test	Mix 2 mL of mouthwash with 2 mL of chloroform and carefully add concentrated sulfuric acid along the side of the test tube.

VII. RESULT

Evaluation Parameters :

1. Organoleptic Evaluation :

Table 4: Organoleptic Evaluation of the Prepared Polyherbal Mouthwash

Sr.no.	Parameters	Observation
1	Colour	Yellowish-green
2	Odor	Pleasant Aromatic
3	Taste	Slightly Bitter and Refreshing
4	Appearance	Smooth and Uniform
5	Clarity	Clear

2. pH Determination :

The pH of the prepared polyherbal mouthwash was determined using pH paper. The pH value of the formulation was found to be 6.4.



3. Homogeneity Test :

The prepared polyherbal mouthwash was homogeneous, showing uniform distribution of ingredients without aggregation.

4. Clarity Test :

The prepared polyherbal mouthwash was evaluated for clarity and was found to be clear and free from suspended particles. The formulation exhibited good transparency and acceptable appearance.

5. Shake Test :

The prepared polyherbal mouthwash was subjected to a shake test and was found to be stable after shaking. No precipitation or phase separation was observed, indicating good physical stability.



Fig no.5:- Organoleptic Test



Fig no.6:- pH Test



Fig no.7:- Homogeneity Test



Fig no.8:- Clarity Test



Fig no.9:- Shake Test

6. Antimicrobial Study :

The prepared polyherbal mouthwash showed antimicrobial activity against the tested oral microorganisms, as indicated by the formation of zones of inhibition.

7. Irritancy Test :

The prepared polyherbal mouthwash was evaluated for irritancy and was found to be non-irritant. No redness, swelling, or discomfort was observed during the study.

8. Viscosity Determination :

The formulation exhibited acceptable flow properties and was easy to pour and handle during evaluation.

9. Stability Study :

The formulation remained physically stable during the reported storage period, with no significant changes in colour, odour, appearance, pH, or clarity.



Phytochemical Screening Test :

Table 5: Results of Phytochemical Screening of the Polyherbal Mouthwash

Sr.no	Test	Observation	Result
1	Alkaloids	Orange precipitate observed	Present
2	Flavonoids	Yellow coloration observed	Present
3	Tannins	Blue-black coloration observed	Present
4	Phenolic Compounds	Dark green coloration observed	Present
5	Glycosides	Brown ring observed	Present
6	Terpenoids	Reddish-brown coloration observed	Present

VIII. DISCUSSION

The present study focused on the formulation and evaluation of a polyherbal mouthwash containing Neem and Tulsi extracts along with clove oil and peppermint oil. The prepared formulation exhibited a yellowish-green colour, pleasant aromatic odour, slightly bitter and refreshing taste, and smooth and uniform appearance. It was also found to be clear and homogeneous, with no precipitation or phase separation observed during the shake test, indicating satisfactory physical characteristics. The pH of the formulation was found to be 6.4, providing a useful physicochemical characteristic of the developed oral formulation. The formulation also demonstrated suitable flow properties during viscosity evaluation. During the stability evaluation, no significant changes in colour, odour, appearance, pH, or clarity were observed under the reported storage conditions. The prepared mouthwash showed antimicrobial activity against the tested oral microorganisms, as indicated by the formation of zones of inhibition. No observable redness, swelling, or discomfort was reported during the irritancy evaluation. These findings provide preliminary support for further investigation of the developed polyherbal formulation. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, glycosides, and terpenoids. The presence of these phytochemical classes indicates the occurrence of various plant-derived constituents in the formulation. However, qualitative phytochemical screening does not establish the concentration or individual contribution of these constituents to the observed antimicrobial activity.

Overall, the developed polyherbal mouthwash demonstrated satisfactory preliminary organoleptic and physicochemical characteristics along with observed antimicrobial activity and the presence of selected phytochemical constituents. Further studies involving quantitative antimicrobial evaluation, extended stability testing, quantitative phytochemical analysis, and appropriate safety and efficacy assessment may provide additional evidence regarding the formulation.

IX. CONCLUSION

The present study successfully formulated a polyherbal mouthwash containing Neem and Tulsi extracts along with clove oil and peppermint oil. The prepared formulation exhibited satisfactory organoleptic and physicochemical characteristics, with a pH of 6.4, good homogeneity and clarity, and no observable phase separation during the shake test. The formulation showed antimicrobial activity against the tested oral microorganisms and demonstrated no observable irritation under the reported test conditions. Preliminary phytochemical screening indicated the presence of alkaloids, flavonoids, tannins, phenolic compounds, glycosides, and terpenoids. Overall, the findings indicate that the developed formulation possesses satisfactory preliminary characteristics and warrants further investigation through detailed antimicrobial, stability, safety, and efficacy studies.

REFERENCES

1. Gupta D, Bhaskar DJ, Gupta RK. Mouthwashes and their role in oral healthcare. International Journal of Advanced Health Sciences. 2014;1(8):5-13.
2. Sharma S, Malhotra R. Herbal mouthwash: A review. International Journal of Pharmaceutical Sciences and Research. 2018;9(7):2654-2660.



3. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Carranza's Clinical Periodontology. 12th ed. Elsevier; 2015.
4. Hiremath SS. Textbook of Preventive and Community Dentistry. 2nd ed. Elsevier; 2011.
5. Samaranayake L. Essential Microbiology for Dentistry. 4th ed. Churchill Livingstone; 2012.
6. Marsh PD. Dental plaque as a microbial biofilm. Caries Research. 2004;38(3):204-211.
7. Tortora GJ, Derrickson B. Principles of Anatomy and Physiology. 15th ed. Wiley Publications; 2017.
8. Ross MH, Pawlina W. Histology: A Text and Atlas. 7th ed. Wolters Kluwer; 2015.
9. Lachman L, Lieberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy. 3rd ed. CBS Publishers; 2013.
10. Aulton ME. Pharmaceutics: The Science of Dosage Form Design. 4th ed. Elsevier; 2018.
11. Sohi H, Sultana Y, Khar RK. Mouthwashes: Current formulations and future prospects. International Journal of Pharmaceutical Sciences Review and Research. 2011;9(2):98-104.
12. Mahajan SD, Doijad CR, Dhanvij SR. Formulation and evaluation of herbal mouthwash for oral healthcare. International Journal of Pharmaceutical Sciences Review and Research. 2021;68(1):45-50.
13. Jain S, Sharma R. Formulation and evaluation of polyherbal mouthwash. Journal of Drug Delivery and Therapeutics. 2022;12(4):98-104.
14. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of Neem (Azadirachta indica). Current Science. 2002;82(11):1336-1345.
15. Subapriya R, Nagini S. Medicinal properties of Neem leaves. Current Medicinal Chemistry. 2005;5(2):149-156.
16. Prashant GM, Chandu GN, Murulikrishna KS, Shafiulla MD. Effect of Neem extract on organisms causing dental caries. Indian Journal of Dental Research. 2007;18(4):148-151.
17. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 54th ed. Nirali Prakashan; 2014.
18. Trease GE, Evans WC. Pharmacognosy. 16th ed. Saunders Elsevier; 2009.
19. Pattanayak P, Behera P, Das D, Panda SK. Ocimum sanctum Linn. A reservoir plant for therapeutic applications. Pharmacognosy Reviews. 2010;4(7):95-105.
20. Cohen MM. Tulsi – Ocimum sanctum: A herb for all reasons. Journal of Ayurveda and Integrative Medicine. 2014;5(4):251-259.
21. Kothari SK, Bhattacharya AK, Ramesh S. Essential oil yield and quality of Ocimum sanctum. Journal of Essential Oil Research. 2004;16(4):336-340.
22. Chaieb K, Hajlaoui H, Zmantar T, et al. The chemical composition and biological activity of clove essential oil. Phytotherapy Research. 2007;21(6):501-506.
23. Cortés-Rojas DF, de Souza CRF, Oliveira WP. Clove (Syzygium aromaticum): A precious spice. Asian Pacific Journal of Tropical Biomedicine. 2014;4(2):90-96.
24. Parle M, Khanna D. Clove: A champion spice. International Journal of Research in Ayurveda and Pharmacy. 2011;2(1):47-54.
25. Arthi S, Ravi V, Kannan R. Evaluation of antimicrobial activity of polyherbal mouthwash. Journal of Population Therapeutics and Clinical Pharmacology. 2023;30(10):2231-2238.
26. McKay DL, Blumberg JB. A review of the bioactivity and potential health benefits of peppermint tea (Mentha piperita L.). Phytotherapy Research. 2006;20(8):619-633.
27. Singh R, Shushni MAM, Belkheir A. Antibacterial and antioxidant activities of Mentha piperita essential oil. Arabian Journal of Chemistry. 2015;8(3):322-328.

