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FORMULATION AND IN VITRO ASSESSMENT OF POLYHERBAL ORAL RINSE USING AZADIRACHTA INDICA, SYZYGIIUM AROMATICUM, AND OCIMUM SANCTUM EXTRACTS AGAINST GINGIVITIS INFECTION

Bharat Chouhan^{*1}, Chandrakant Mod², Rajat Pawar³

¹Department of Pharmaceutical Chemistry, Assistant Professor, Acharya Rajendra Suri Shiksha Mahavidhyalaya (ARSSM) College of Pharmacy, Mandsaur (M.P.), 458002

²Department of Pharmaceutics, Associate Professor, Acharya Rajendra Suri Shiksha Mahavidhyalaya (ARSSM) College of Pharmacy, Mandsaur (M.P.), 458002

³Department of Pharmaceutics, Associate Professor, Swami Vivekanand College of Pharmacy, Indore, (M.P.), 452020

Corresponding author: Bharat Chouhan*, E-mail: pharm06bharat@gmail.com,

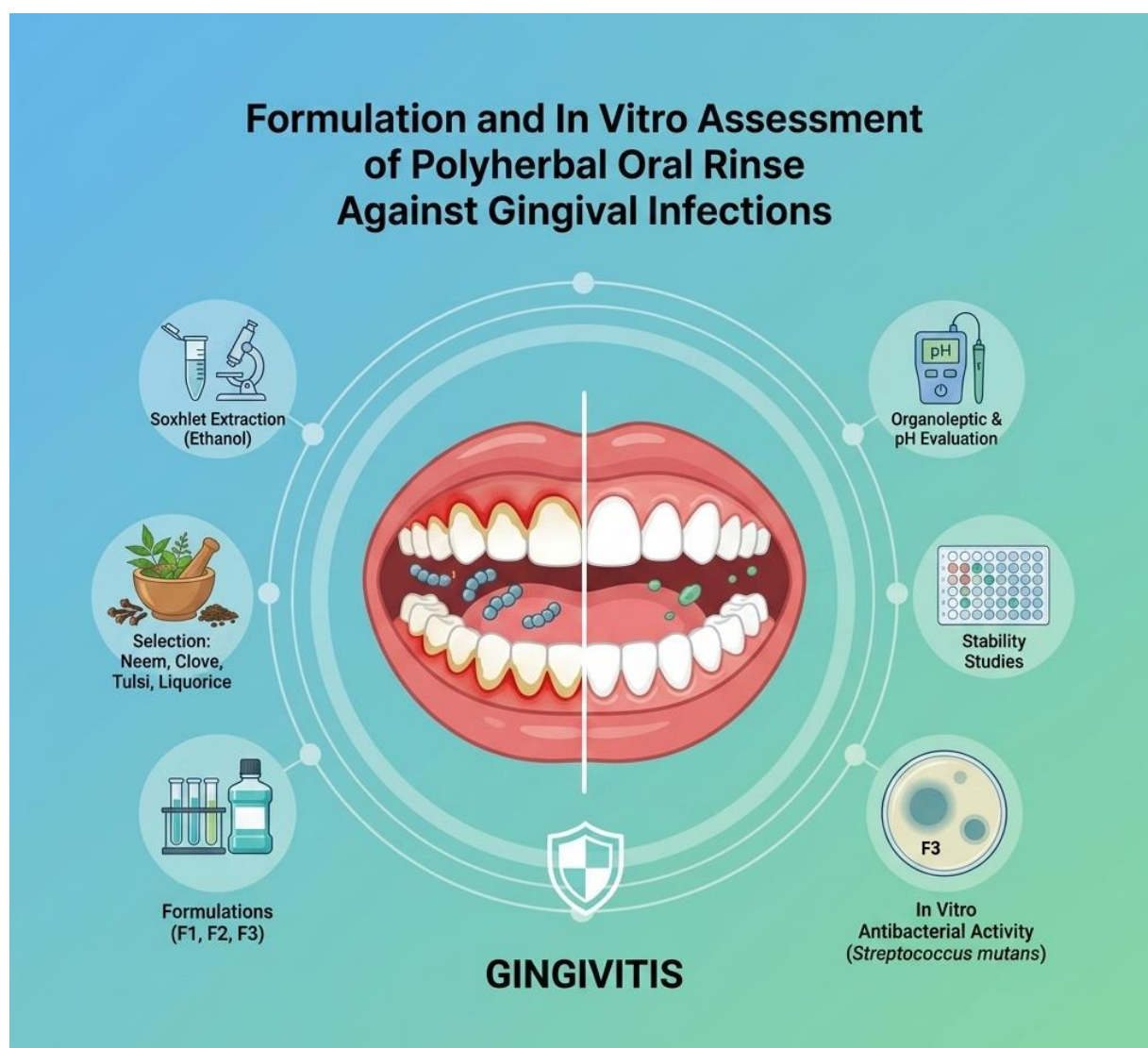
Orcid ID: <https://orcid.org/0009-0002-2902-1684>

ABSTRACT

The present study focuses on the formulation and in vitro evaluation of a polyherbal mouthwash for the management of gingivitis and other oral health problems. Herbal ingredients such as neem (*Azadirachta indica*), clove (*Syzygium aromaticum*), tulsi (*Ocimum sanctum*), and liquorice (*Glycyrrhiza glabra*) were selected due to their well-known antimicrobial, anti-inflammatory, and therapeutic properties. The plant materials were extracted using ethanol by Soxhlet extraction method and incorporated into three different formulations (F1, F2, and F3) with varying concentrations. Excipients such as menthol, propylene glycol, Tween 80, sodium benzoate, and distilled water were used to prepare the mouthwash. The prepared formulations were evaluated for organoleptic properties, pH, stability, and antibacterial activity against *Streptococcus mutans*. All formulations showed acceptable physical characteristics, stability, and pH within the suitable range for oral use. Among them, formulation F3 exhibited the highest antibacterial activity with the largest zone of inhibition. The results suggest that the polyherbal mouthwash possesses significant antimicrobial activity and can serve as a safe, effective, and economical alternative to conventional chemical mouthwashes for maintaining oral hygiene and preventing gingivitis.

Keywords: Polyherbal mouthwash, Gingivitis, Antibacterial activity, *Azadirachta indica*, *Streptococcus mutans*.

GRAPHICAL ABSTRACT



INTRODUCTION

Dental diseases are major global public health concerns, with caries and periodontal disorders among the most prevalent conditions. Medicinal plants are widely used in traditional therapy due to their affordability and fewer side effects. *Ocimum sanctum* shows significant therapeutic potential, especially for managing common oral diseases in underserved populations worldwide.^[1]

Dental plaque is recognized as the primary etiological factor in initiating gingivitis and its progression to periodontitis.^[2] Mechanical plaque control remains an effective method for preventing dental caries and periodontal diseases.^[3] Mouthrinses act as adjuncts by delivering active agents that inhibit bacterial adhesion and biofilm formation. Maintaining oral health requires proper hygiene, fluoride use, and regular dental visits.^[4] Chemical antimicrobial agents further help reduce biofilm formation by inhibiting bacterial growth and metabolism.^[4,5,6] Various agents, including chlorhexidine gluconate, essential oils, and others, have been evaluated, with chlorhexidine being the most effective antiseptic.^[7-9] However, its use is associated with side effects like taste alteration and tooth staining, and its role in caries prevention remains uncertain.^[10-12] Natural herbs such as basil, neem, clove oil, and mint, used individually or in combination, have been scientifically shown to be safe and effective in managing oral health problems like bleeding gums, mouth ulcers, and tooth decay, offering

therapeutic benefits with minimal or no side effects ^[13]. Therefore, the World Health Organization has recommended exploring natural plant-based alternatives.

MATERIALS AND METHODS

Materials

The herbal ingredients selected for the formulation of polyherbal mouthwash included neem (*Azadirachta indica*), clove (*Syzygium aromaticum*), tulsi (*Ocimum sanctum*), and liquorice (*Glycyrrhiza glabra*). These crude drugs were procured from the local market and authenticated before use. The supporting excipients used in the formulation were menthol, propylene glycol, Tween 80, sodium benzoate, and distilled water.

The chemical constituents and functions of ingredients were considered during formulation. Clove contains eugenol which acts as an analgesic and antimicrobial agent, neem possesses antiseptic and anti-plaque activity, tulsi exhibits antimicrobial and antioxidant properties, while liquorice acts as a soothing and sweetening agent. Menthol was added for its cooling effect, propylene glycol served as a humectant and solvent, Tween 80 functioned as an emulsifying agent, and sodium benzoate was used as a preservative. Distilled water was used as the vehicle.

Preparation of Plant Extracts

The collected plant materials were first washed thoroughly with water to remove impurities and then dried under shade to preserve their active constituents. After complete drying, the materials were coarsely powdered using a mechanical grinder.

Each powdered sample (neem, clove, tulsi, and liquorice) was subjected to Soxhlet extraction using ethanol as a solvent. Approximately 300 mL of ethanol was used for each sample, and extraction was carried out for 6 hours. After extraction, the solutions were filtered using Whatman filter paper to remove solid residues.

The filtrates obtained were then concentrated by evaporating the solvent on a water bath maintained at 60–80°C until a semi-solid extract was obtained. These extracts were stored in airtight containers for further use. The individual extracts were also preliminarily evaluated for antimicrobial activity using the zone of inhibition method.

Formulation of Polyherbal Mouthwash

Three different formulations (F1, F2, and F3) were prepared by varying the concentration of herbal extracts. The composition of each formulation per 100 mL is given below:

- Clove extract: 0.3 g (F1), 0.6 g (F2), 0.9 g (F3)
- Neem extract: 0.2 g (F1), 0.4 g (F2), 0.6 g (F3)
- Tulsi extract: 0.2 g (F1), 0.4 g (F2), 0.6 g (F3)
- Liquorice extract: 0.3 g (F1), 0.6 g (F2), 0.9 g (F3)
- Menthol: 1 g
- Propylene glycol: 10 mL
- Tween 80: 5 mL
- Sodium benzoate: 1 g
- Distilled water: Quantity sufficient (q.s.) to 100 mL

Procedure for Mouthwash Preparation

The accurately weighed herbal extracts were first mixed with propylene glycol in a clean glass beaker to form a uniform mixture. The mixture was then heated to approximately 60°C on a water bath.

Subsequently, Tween 80 and menthol were added to the mixture with continuous stirring using a magnetic stirrer at around 300 rpm to ensure proper emulsification and uniform dispersion of ingredients.

In a separate container, sodium benzoate was dissolved in a small quantity of distilled water and then added to the main mixture. The entire solution was stirred continuously to achieve homogeneity.

Finally, distilled water was added gradually to make up the volume to 100 mL, and the mixture was stirred until a clear and uniform mouthwash solution was obtained. The prepared formulations were transferred into suitable containers and stored at room temperature for further evaluation.

Evaluation Parameters

Organoleptic (Morphological) Evaluation

The prepared formulations were visually examined for color, odor, taste, and physical form. Observations were recorded over a period of one week to assess acceptability.

pH Determination

The pH of each formulation was measured using a calibrated digital pH meter. The instrument was standardized using buffer solutions of pH 4 and pH 7 before measurement. The pH was recorded initially and monitored over a period of 7 weeks to assess stability.

Stability Studies

Stability testing was performed using a freeze–thaw cycle method. Each formulation was subjected to three cycles of storage at 4°C and 40°C, with each condition maintained for 48 hours. The formulations were observed for any changes in appearance, phase separation, or precipitation.

Antibacterial Activity

The antibacterial activity of the formulations was evaluated using the disc diffusion method against *Streptococcus mutans*. Nutrient agar plates were prepared and inoculated with bacterial suspension to form a uniform lawn.

Discs impregnated with the mouthwash formulations were placed on the agar surface and incubated at appropriate conditions. After incubation, the zone of inhibition around each disc was measured in millimeters. The results were compared among the three formulations to determine the most effective preparation.

RESULTS AND DISCUSSION

The prepared polyherbal mouthwash formulations (F1, F2, and F3) were evaluated for various physicochemical and antimicrobial parameters. The results obtained from the experimental studies are presented below in a systematic manner.

Organoleptic (Morphological) Evaluation

The organoleptic properties of the formulations were assessed based on color, odor, taste, and physical form. All three formulations were found to be liquid in nature with acceptable sensory characteristics. Slight variations in color intensity were observed due to differences in the concentration of herbal extracts.

Table 1: Organoleptic Evaluation of Polyherbal Mouthwash Formulations

S. No.	Parameter	F1	F2	F3
1	Color	Moss green	Olive green	Army green
2	Odor	Minty	Minty	Minty
3	Taste	Refreshing mint	Refreshing mint	Refreshing mint
4	Physical Form	Liquid	Liquid	Liquid

All formulations exhibited a pleasant minty odor and refreshing taste due to the presence of menthol. The color variation from moss green to army green indicates increased concentration of herbal extracts from F1 to F3. No phase separation or turbidity was observed, indicating good formulation stability.

pH Determination

The pH of mouthwash is an important parameter for ensuring compatibility with oral mucosa. The pH of all formulations was measured over a period of 7 weeks to evaluate stability.

Table 2: pH Variation of Formulations During Storage

Week	F1	F2	F3
Week 1	5.00	5.50	6.00
Week 2	4.90	5.30	5.75
Week 3	4.70	5.10	5.64
Week 4	4.50	4.85	5.42
Week 5	4.40	4.62	5.18
Week 6	4.20	4.31	4.80
Week 7	4.12	4.15	4.56

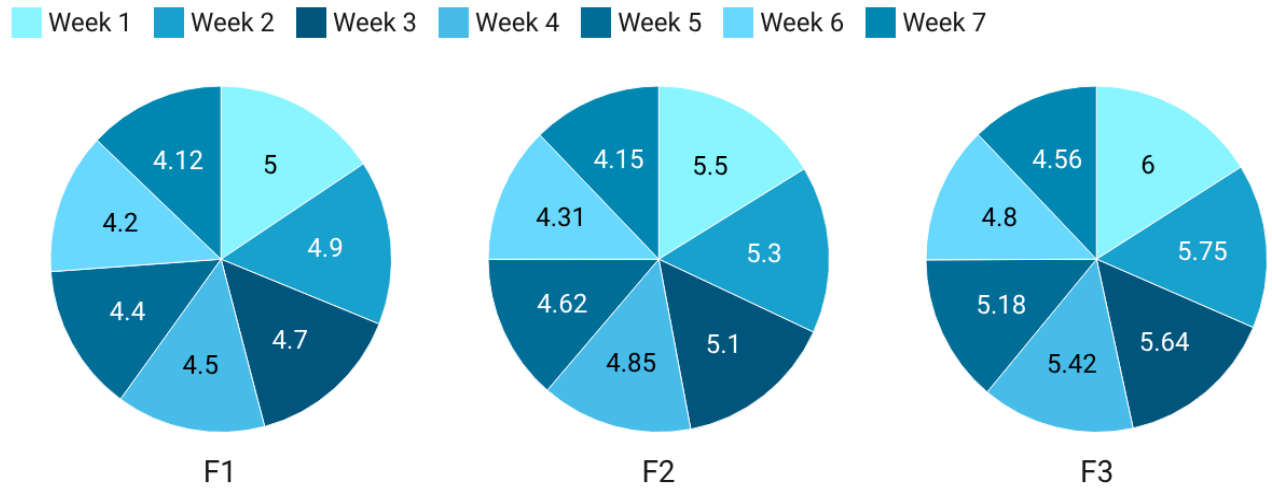


Figure 1: pH Variation of Formulations During Storage

The pH of all formulations showed a gradual decrease over time. However, the pH remained within the acceptable range (4–6) for oral preparations. Among the three, F3 maintained a relatively higher pH throughout the study, indicating better stability and suitability for oral use.

Stability Studies

The stability of the prepared formulations was evaluated using the freeze–thaw cycle method. The formulations were subjected to alternating temperature conditions (4°C and 40°C) for three cycles.

Table 3: Stability Study of Polyherbal Mouthwash Formulations

S. No.	Parameter	F1	F2	F3
1	Phase Separation	Absent	Absent	Absent
2	Precipitation	Absent	Absent	Absent
3	Color Change	No change	No change	No change
4	Odor Change	No change	No change	No change

All formulations remained stable under stress conditions. No phase separation, precipitation, or changes in organoleptic properties were observed, indicating good physical stability of the prepared mouthwash.

Antibacterial Activity

The antibacterial activity of the polyherbal mouthwash formulations was evaluated against *Streptococcus mutans* using the disc diffusion method. The effectiveness of each formulation was determined by measuring the zone of inhibition.

Table 4: Antibacterial Activity of Polyherbal Mouthwash (Zone of Inhibition in mm)

Formulation	Zone of Inhibition (mm)
F1	10 ± 2
F2	13 ± 2
F3	15 ± 2

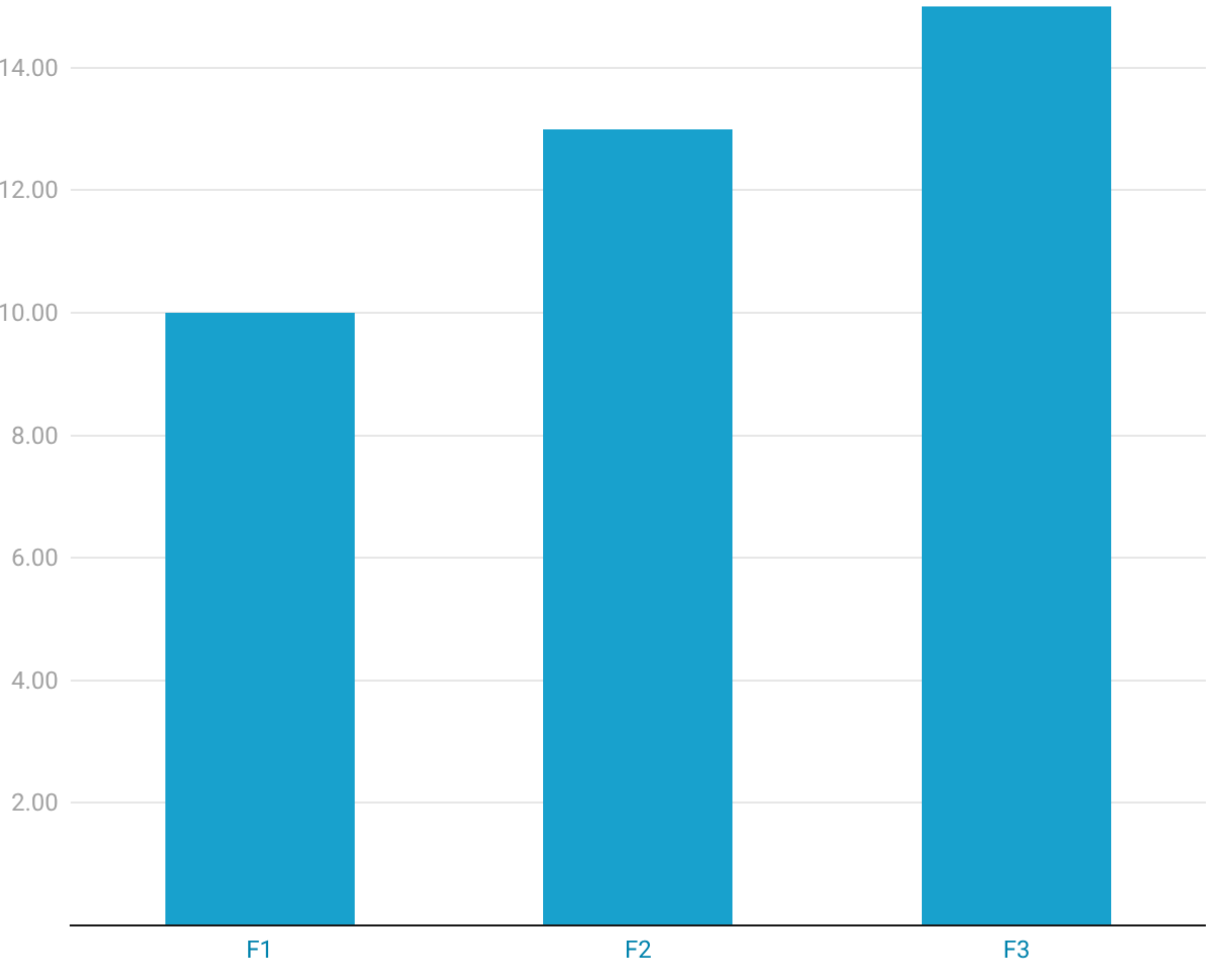


Figure 2: Antibacterial Activity of Polyherbal Mouthwash (Zone of Inhibition in mm)

The antibacterial activity increased with the increase in concentration of herbal extracts. Among all formulations, F3 exhibited the highest zone of inhibition (15 ± 2 mm), indicating superior antimicrobial activity against *Streptococcus mutans*. F2 showed moderate activity, while F1 showed the least antibacterial effect.

Comparative Evaluation of Formulations

A comparative assessment of all formulations was performed based on physicochemical properties and antimicrobial activity.

Table 5: Comparative Evaluation of Polyherbal Mouthwash Formulations

Parameter	F1	F2	F3
Organoleptic Properties	Good	Good	Excellent
pH Stability	Moderate	Good	Excellent
Physical Stability	Stable	Stable	Stable
Antibacterial Activity	Low	Moderate	High

The comparative evaluation indicates that formulation F3 performed better in almost all evaluation parameters. It showed excellent organoleptic properties, better pH stability, and the highest antibacterial activity.

Overall Findings

The study demonstrated that the polyherbal mouthwash formulations prepared using neem, clove, tulsi, and liquorice extracts possess desirable physicochemical and antimicrobial properties. The gradual increase in herbal extract concentration from F1 to F3 resulted in enhanced therapeutic efficacy.

The organoleptic evaluation confirmed that all formulations were acceptable in terms of taste, odor, and appearance, which is important for patient compliance. The pH studies indicated that the formulations are suitable for oral use and remain stable over time.

The stability studies further confirmed that the formulations can withstand temperature variations without undergoing any physical or chemical degradation. This suggests good shelf-life potential for the developed product.

The antibacterial study clearly showed that the polyherbal formulation is effective against *Streptococcus mutans*, a major causative organism of dental caries and gingivitis. The enhanced activity observed in F3 can be attributed to the higher concentration of active phytoconstituents such as eugenol (from clove), azadirachtin (from neem), and other antimicrobial compounds.

CONCLUSION

The study successfully formulated and evaluated a polyherbal mouthwash containing neem, clove, tulsi, and liquorice extracts. All formulations demonstrated good physicochemical properties, stability, and acceptable pH for oral use. The antibacterial studies confirmed effective activity against *Streptococcus mutans*, with formulation F3 showing superior

performance. The presence of natural herbal ingredients contributes to reduced side effects and enhanced safety compared to synthetic mouthwashes. Overall, the developed formulation shows promising potential as an economical and effective alternative for the prevention and management of gingivitis and other oral infections. Further clinical studies are recommended to validate its therapeutic efficacy.

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