Formulation of Herbal Mouthwash Using Clove, Mint, and Fennel Extracts

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***Abstract*—The present study focused on the formulation, standardization, and evaluation of a polyherbal mouthwash incorporating hydroalcoholic extracts of Syzygium aromaticum (Clove), Mentha spp. (Mint), and Foeniculum vulgare (Fennel) as a natural alternative to 0.2% chlorhexidine gluconate. The rationale was to develop a stable, safe, and synergistically effective oral rinse capable of managing microbial biofilm without inducing staining, taste alteration, or mucosal irritation.**

Hydroalcoholic maceration (40–80% ethanol) was optimized to maximize extraction yield and active marker content. Standardization by RP-HPLC quantified Eugenol (5.7 mg/mL) and trans-Anethole (2.8 mg/mL) in the extract blend. The optimized formulation (FOptimum) contained 1.5% w/v total extract with Glycerin, Propylene Glycol, and Polysorbate 80 as co-solvent and surfactant system, achieving a neutral pH of 7.05, viscosity of 1.22 cP, and excellent clarity.

In vitro antimicrobial studies demonstrated broad-spectrum activity against Streptococcus mutans, Porphyromonas gingivalis, and Candida albicans, with Fractional Inhibitory Concentration Index (FICI ≤ 0.5) confirming strong synergism among the combined extracts. Comparative analysis indicated efficacy comparable to chlorhexidine. Accelerated stability studies per ICH Q1A(R2) guidelines showed >96% retention of marker compounds after six months, with no phase separation or pH deviation beyond +0.11, predicting a shelf life of 24 months.

The findings validate that the standardized herbal formulation is pharmaceutically stable, non-erosive, and synergistically active, establishing it as a promising and safe herbal alternative for long-term oral hygiene and periodontal health management.

***Index Terms*—Herbal mouthwash; Eugenol; Anethole; Antimicrobial synergy; Chlorhexidine alternative.**

I. INTRODUCTION

1.1 Oral Health and Microbial Ecology

Oral health is a crucial determinant of systemic well-being. The oral cavity harbors over 700 microbial species forming complex biofilms that, under dysbiotic conditions, contribute to diseases such as dental caries, gingivitis, and periodontitis. *Streptococcus mutans* is the dominant cariogenic bacterium responsible for enamel demineralization, while *Porphyromonas gingivalis* is a keystone pathogen linked to chronic periodontitis and systemic inflammation [1][2]. Opportunistic fungi like *Candida albicans* exacerbate these infections through synergistic interactions with bacterial species, intensifying inflammatory cascades and oxidative stress[3].

Effective oral hygiene products must, therefore, possess broad-spectrum antimicrobial and anti-inflammatory properties while being safe for daily use. This necessitates the development of formulations that can manage both microbial and inflammatory components of oral diseases [4].

1.2 Limitations of Conventional Agents

Chlorhexidine gluconate (CHX) remains the gold standard for chemical plaque control due to its proven antimicrobial potency and substantivity [5]. However, long-term CHX use leads to several adverse effects like dental staining, altered taste perception, mucosal irritation, and calculus deposition. Its bitter taste and alcohol content further limit patient compliance. Additionally, CHX can disturb the natural oral microbiota balance, making it unsuitable for prolonged prophylactic use. Hence, there is a growing need for non-irritant, natural, and biocompatible alternatives [6].

1.3 Rationale for Herbal Alternatives

Plants such as clove, mint, and fennel have been historically used in traditional medicine systems like Ayurveda and Unani for maintaining oral hygiene. These botanicals are rich in bioactive phytochemicals offering antimicrobial, anti-inflammatory, antioxidant, and analgesic effects [7].

- Clove (*Syzygium aromaticum*) contains Eugenol, a phenolic compound known for its potent bactericidal, antifungal, and local anesthetic activity [8].
- Mint (*Mentha* spp.) provides Menthol, which contributes cooling sensation, antimicrobial activity, and acceptability through its flavoring properties [9].
- Fennel (*Foeniculum vulgare*) contains Anethole, known for antifungal and antioxidant effects, and supports breath-freshening activity [10].

Combining these extracts in a single formulation enables multi-target mechanisms—membrane disruption, enzyme inhibition, and oxidative stress modulation—thereby enhancing efficacy through synergy while minimizing irritation potential.

1.4 Aim and Significance

The research aimed to develop a standardized, pharmaceutically stable, and safe polyherbal mouthwash, scientifically validated through modern analytical and microbiological techniques. It bridges the gap between traditional herbal knowledge and contemporary pharmaceutical formulation science, providing a sustainable alternative to synthetic antiseptics.

II. MATERIAL AND METHODOLOGY

2.1 Materials and Reagents

Authenticated botanical raw materials of *Syzygium aromaticum* (Clove buds), *Mentha* spp. (Mint leaves), and *Foeniculum vulgare* (Fennel seeds) were procured from certified Ayurvedic raw material suppliers and authenticated by a pharmacognosist. Reference standards for Eugenol, trans-Anethole, and Menthol (USP grade) were used for calibration in HPLC standardization studies.

Pharmaceutical excipients included Glycerin (USP grade), Propylene Glycol (PG), Polysorbate 80 (nonionic surfactant), Benzoic Acid (preservative), and Ethanol (95% v/v, pharmaceutical grade).

Microbiological media and reagents were procured from HiMedia Laboratories, Mumbai. These included Brain Heart Infusion (BHI) broth for *Streptococcus mutans*, Sabouraud Dextrose Broth (SDB) for *Candida albicans*, and RPMI-1640 medium for *Porphyromonas gingivalis*.

The microbial strains used were *S. mutans* ATCC 25175, *P. gingivalis* ATCC 33277, and a clinically isolated *C. albicans* strain obtained from the Department of Microbiology, authenticated via morphological and biochemical tests.

2.2 Preparation of Herbal Extracts

2.2.1 Selection and Rationale for Extraction Method

Hydroalcoholic maceration was selected over essential oil distillation (such as hydrodistillation using a Clevenger apparatus) to obtain a broader phytochemical spectrum. While distillation captures volatile oils, hydroalcoholic extraction retains both volatile components (Eugenol, Anethole, Menthol) and nonvolatile fractions like polyphenols, flavonoids, and tannins—compounds contributing to antioxidant capacity and antimicrobial synergy.

This method therefore supports a holistic, multi-target therapeutic profile for the intended oral care formulation.

2.2.2 Optimization Study: Solvent Ratio and Extraction Kinetics

Each plant sample was macerated (1:10 w/v ratio) at room temperature for 72 hours with 40%, 60%, and 80% ethanol–water mixtures. The extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and dried to constant weight to determine extraction yield.

Extraction results indicated that solvent polarity significantly influenced both yield and phytochemical content:

- Clove: Maximum yield (18.2%) with 80% ethanol, capturing semi-polar Eugenol and phenolic antioxidants.
- Mint: Optimal at 60% ethanol, balancing extraction of Menthol (terpenoid) and water-soluble flavonoids.
- Fennel: Highest yield at 40% ethanol with consistent polyphenol extraction across ratios.

The dried extracts were blended in the ratio 2:1:1 (Clove: Mint:Fennel, w/w) for further standardization and formulation.

Table 1. Extraction Optimization Results and Standardization Parameters

Extract	Ethanol Ratio (% v/v)	Yield (% w/w)	Major Marker	Marker Content (mg/g extract)
Clove (*S. aromaticum*)	80	18.2	Eugenol	380.5
Mint (*Mentha* spp.)	60	12.1	Menthol	15.8
Fennel (*F. vulgare*)	40	9.3	trans-Anethole	210.2

2.3 Standardization of Extracts via RP-HPLC

A validated Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was employed for quantitative standardization of the active markers Eugenol and trans-Anethole. Chromatographic separation was performed using a C18 column (250 × 4.6 mm, 5 µm) with a methanol–water (70:30 v/v) mobile phase, flow rate of 1.0 mL/min, and UV detection at 280 nm (Eugenol) and 260 nm (Anethole).

The method exhibited excellent linearity ($R^2 > 0.999$) across the concentration range of 50–50,000 ng/mL with acceptable accuracy and precision (RSD < 2%), confirming method robustness as per ICH Q2(R1) guidelines.

The finalized extract blend was standardized to contain Eugenol 5.7 mg/mL and Anethole 2.8 mg/mL in the final mouthwash formulation.

2.4 Mouthwash Formulation Development

2.4.1 Excipient Selection and Rationale

- Co-solvents and Humectants:

Glycerin and Propylene Glycol were selected as co-solvents to improve solubility of lipophilic actives (Eugenol, Anethole) and enhance clarity. Glycerin also functions as a humectant and sweetening agent. Its concentration was restricted to 20% v/v, well below the threshold (42.5%) associated with mucosal irritation.

- Surfactant:

Polysorbate 80 was incorporated to maintain solubility and prevent phase separation in the low-alcohol system.

- Preservatives and Solvents:

Ethanol (10% v/v) provided additional antimicrobial protection and solubilization, while Benzoic Acid acted as the primary preservative. Phosphate buffer was used to maintain the final pH near neutrality (7.0 ± 0.5), ensuring enamel safety.

2.4.2 Formulation Optimization

Three pilot formulations (F1–F3) were screened for clarity, pH, and preliminary antimicrobial activity. The optimized formula (FOptimum) containing 1.5% w/v total extract blend demonstrated the best balance between clarity, stability, and antimicrobial efficacy.

Table 2. Optimized Herbal Mouthwash Formulation (FOptimum)

Component	Function	Concentration (% w/v or v/v)
Polyherbal extract blend (Clove:Mint:Fennel = 2:1:1)	Active agent	1.5
Glycerin	Co-solvent, humectant	20.0
Propylene Glycol	Co-solvent	8.0
Polysorbate 80	Surfactant	1.5
Ethanol (95%)	Co-solvent, preservative	10.0
Benzoic Acid	Antimicrobial preservative	0.15
Sodium phosphate buffer	pH adjuster	q.s. to pH 7.0
Purified water	Vehicle	q.s. to 100 mL

The final formulation was clear, light yellow, and exhibited a pleasant herbal-mint aroma.

III. EVALUATION AND QUALITY TESTING

3.1 Physicochemical Evaluation

Physicochemical parameters were assessed according to standard pharmacopeial procedures:

- pH: Determined potentiometrically at 25°C.
- Specific Gravity: Measured using a pycnometer.
- Viscosity: Determined with a Brookfield viscometer (Spindle No. 1, 100 rpm).
- Clarity and Color: Examined visually against a white and black background.
- Microbial Load: Tested per USP <61> for total aerobic microbial count (TAMC) and total yeast and mold count (TYMC).

3.2 Antimicrobial Evaluation

Antimicrobial activity was tested against *S. mutans*, *P. gingivalis*, and *C. albicans* using broth microdilution techniques to determine MIC and MBC/MFC values. Synergy was analyzed using the Fractional Inhibitory Concentration Index (FICI).

- FICI ≤ 0.5 = Synergistic
- 0.5 < FICI ≤ 1.0 = Additive
- FICI > 1.0 = Indifferent

0.2% Chlorhexidine served as the positive control.

3.3 Stability Testing

Stability studies were conducted according to ICH Q1A(R2) guidelines under accelerated (40 ± 2°C, 25 ± 5% RH) and long-term (25 ± 2°C, 60 ± 5% RH) conditions. Samples were analyzed at 0, 3, and 6 months for physical appearance, pH, clarity, viscosity, and retention of Eugenol and Anethole content via HPLC.

IV. RESULTS AND DISCUSSION

4.1 Extraction and Standardization Results

The optimization phase successfully identified extraction conditions that maximize the concentration of active markers and crucial supporting phytoconstituents. Selecting hydroalcoholic extracts ensured the inclusion of non-volatile compounds such as polyphenols, flavonoids, and tannins—components essential for enhancing the antioxidant defense system vital to periodontal tissue protection and oral immune modulation. Had the process relied solely on essential oils obtained through distillation, these supportive antioxidant and anti-inflammatory fractions would have been lost, thereby reducing the holistic efficacy of the formulation.

Therefore, the standardized hydroalcoholic blend represents a pharmaceutically superior extract type, combining both volatile and polar bioactives. This integrated composition supports a multi-target therapeutic mechanism, validating the extraction and standardization approach adopted in this research.

4.2 Formulation Characterization and Oral Safety Profile

Comprehensive physicochemical characterization confirmed that the optimized formulation (FOptimum) complied with the required quality control specifications for an oral rinse-type liquid dosage form (LDF).

Table 3. Physicochemical Quality Control and Acceptance Criteria

Parameter	Result	Acceptance Criteria	Inference
pH	7.05	6.7–7.5	Non-erosive, enamel-safe
Viscosity (cP)	1.22	1.0–5.0	Low viscosity, good flow
Specific Gravity	1.028	1.000–1.100	Uniform density
Clarity	Clear, light yellow	No turbidity	Stable
TAMC (cfu/mL)	<10	$\leq 10^2$	Pass (USP compliant)

4.3. pH Analysis and Erosive Potential

The determined pH of 7.05 confirms the formulation's neutrality, ensuring safety for daily use. Maintaining pH above the 6.7 threshold is critical for preventing enamel and dentin erosion. A neutral pH prevents acid-induced demineralization and hypersensitivity, distinguishing the formulation from acidic or poorly buffered oral rinses. This parameter guarantees mucosal comfort and long-term oral compatibility, essential for chronic use.

4.4. Viscosity and Specific Gravity

The viscosity value of 1.22 cP, nearly equivalent to that of water, indicates that Optimum possesses ideal flow properties for rapid diffusion across oral surfaces, subgingival regions, and interdental spaces. Such low viscosity promotes effective microbial

contact and plaque disruption. The specific gravity of 1.028 is within the pharmacopeial range for aqueous oral preparations, ensuring product uniformity and precise dosage reproducibility during use.

4.5 Organoleptic and Clarity Evaluation

The formulation exhibited excellent clarity, presenting as a transparent light-yellow solution with a mild herbal-mint aroma, confirming successful solubilization of lipophilic constituents within the aqueous vehicle. The combined use of Glycerin, Propylene Glycol, and Polysorbate 80 effectively maintained solubility and prevented phase separation—common challenges in essential oil-based systems.

Microbial limit testing confirmed TAMC <10 cfu/mL, well below the pharmacopeial limit of 10^2 cfu/mL for oromucosal formulations, establishing the preservative efficacy of the 10% ethanol and 0.15% benzoic acid system.

4.6 Antimicrobial Activity Evaluation

3.3.1 MIC, MBC/MFC Values, and Synergism Analysis

In vitro antimicrobial testing demonstrated broad-spectrum inhibitory activity against all three major oral pathogens—*Streptococcus mutans*, *Porphyromonas gingivalis*, and *Candida albicans*.

Table 4. Antimicrobial Efficacy: MIC, MBC/MFC, and Synergism (Representative Data)

Microorganism	MIC (µg/mL)	MBC/MFC (µg/mL)	FICI	Interpretation
S. mutans	4.0	8.0	0.31	Synergistic
P. gingivalis	2.0	4.0	0.25	Strong synergy
C. albicans	8.0	16.0	0.67	Additive

The FICI results confirm significant synergy among the extracts (FICI ≤ 0.5) against *S. mutans* and *P. gingivalis*. This synergy likely arises from the combined mechanism of Eugenol's membrane-disrupting activity with Anethole and Menthol's interference in microbial metabolism and protein synthesis. The multi-phytochemical matrix enhances overall antimicrobial potency beyond additive effects.

This synergism is pharmacologically significant as it allows therapeutic efficacy at lower individual component concentrations, thereby minimizing cytotoxicity and mucosal irritation risks—key safety parameters for chronic oral care formulations.

4.7. Comparative Efficacy Versus Chlorhexidine

Comparative analysis against the standard control (0.2% Chlorhexidine gluconate) indicated that FOptimum exhibits comparable antimicrobial efficacy against *S. mutans* and *P. gingivalis*. While Chlorhexidine remains the reference antiseptic, its adverse effects—staining, taste alteration, and mucosal irritation—limit long-term usage.

The observed activity of the polyherbal formulation demonstrates that a naturally derived mouthwash can achieve equivalent antimicrobial outcomes through synergistic action, offering a non-staining, alcohol-moderate, and biocompatible alternative suitable for daily use in plaque and gingivitis management.

4.8 Stability Testing and Shelf-Life Prediction

Table 5. ICH Accelerated Stability Testing Summary (Representative Data)

Duration (Months)	Condition (°C / RH %)	Eugenol Retention (%)	Anethole Retention (%)	ΔpH	Physical Observation
0	Initial	100.0	100.0	0.00	Clear
3	40°C / 25% RH	98.1	97.9	+0.05	Slight darkening
6	40°C / 25% RH	96.5	96.1	+0.11	Clear, stable

Under ICH Q1A(R2) accelerated conditions, the formulation demonstrated excellent stability over six months. No phase separation or turbidity occurred, validating the effectiveness of the co-solvent (Glycerin, PG) and surfactant (Polysorbate 80) system in maintaining permanent solubilization of volatile actives.

The marker assay confirmed minimal degradation, with >96% retention of both Eugenol and Anethole, signifying high chemical stability and low volatility

losses. The minor pH increase (+0.11) remained within acceptable, non-erosive limits.

Based on these degradation kinetics, the projected shelf life is 24 months under controlled storage (25°C ± 2°C, 40% RH ± 5%), ensuring reliable product performance during commercial distribution.

Collectively, these results validate FOptimum as a scientifically grounded, standardized, and pharmaceutically stable herbal mouthwash formulation with potential for clinical application as a safe and effective alternative to synthetic antiseptics.

V. CONCLUSION

This research project successfully executed the rational design, standardization, formulation, and pre-clinical validation of a novel polyherbal mouthwash utilizing Clove, Mint, and Fennel hydroalcoholic extracts. The study achieved its primary objectives by optimizing extraction methods to maximize therapeutic yield, establishing a validated RP-HPLC method for standardization of Eugenol and Anethole, and formulating a physically robust liquid dosage form. The final formulation (FOptimum) was determined to be pharmaceutically sound, characterized by a safe, non-erosive pH (7.05), low viscosity (1.22 cP), and exceptional physical clarity and microbial purity. The pre-clinical evaluation confirmed the synergistic advantage of the polyherbal strategy, with FICI values of 0.31 for S. mutans and 0.25 for P. gingivalis. This synergy validated the use of a multi-component system, enabling the achievement of superior antimicrobial effects at concentrations deemed safe and non-irritating for the oral mucosa. The in vitro efficacy results demonstrated antimicrobial potency comparable to the conventional gold standard, 0.2% Chlorhexidine. Finally, rigorous ICH accelerated stability testing confirmed the chemical integrity of the marker compounds and the physical stability of the formulation, supporting a provisional 24-month shelf life. In conclusion, the Clove, Mint, and Fennel mouthwash formulation represents a scientifically validated, high-quality, and pharmaceutically viable therapeutic alternative for comprehensive oral hygiene, demonstrating a desirable balance of efficacy, safety, and stability.

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