JOURNAL OF PHARMA INSIGHTS AND RESEARCH ISSN NO. 3048-5428

RESEARCH ARTICLE

Development and Physicochemical Evaluation of a Polyherbal Gargle

SS Prasanna Kumar Ponnaganti*, Venkata Nagapushpa Mounika Alladi, Mohan Pothu Raju Putti, Sailaja Santhati, Nirmala Sruthi Tirumala, Hema Vippili

Department of Pharmaceutics, A.K.R.G College of Pharmacy, Nallajerla, Andhra Pradesh, India

Publication history: Received on 22nd January 2026; Revised on 3rd March 2026; Accepted on 4th March 2026

Article DOI: 10.69613/84shj143

Abstract: Oral health maintenance is regarded as a critical barrier against systemic pathologies, yet the prolonged use of synthetic oral rinses often precipitates adverse effects such as tooth staining and mucosal desensitization. A polyherbal gargle was developed using bioactive extracts from *Nyctanthes arbor-tristis* (Parijat), *Piper betle* (Betel), *Psidium guajava* (Guava), and *Ocimum sanctum* (Tulsi), supplemented with natural flavoring and antimicrobial agents like clove, ginger, and turmeric. Four distinct formulations (F1–F4) were prepared by varying the ratios of primary herbal extracts in an aqueous-glycerin base. Physicochemical characterization revealed stable brown liquid formulations with pH values ranging from 6.65 ± 0.03 to 6.95 ± 0.03, aligning with the physiological environment of the oral cavity. Specific gravity and viscosity measurements indicated superior consistency in the polyherbal blend (F4) compared to single-extract variations. Safety profiles were established through acute dermal and mucosal irritancy studies in New Zealand White albino rabbits showing no signs of erythema or edema over a 72-hour observation period. Antimicrobial efficacy, evaluated via the cup plate method against mixed oral microflora, demonstrated significant zones of inhibition for all batches, with the synergistic combination in F4 exhibiting the highest potency. Stability studies conducted over 45 days at ambient conditions confirmed the physical and chemical integrity of the gargle. These results indicate that the optimized polyherbal gargle (F4) offers a safe, effective, and standardized botanical alternative to conventional chemotherapeutic mouthwashes for the management of oropharyngeal infections.

Keywords: Phytotherapy; Polyherbal Gargle; Antimicrobial Activity; Oral Hygiene; Physicochemical Evaluation.

1. Introduction

Optimal oral hygiene is a fundamental prerequisite for preventing periodontal diseases, dental caries, and halitosis, while also mitigating the risk of systemic complications such as cardiovascular disease and diabetes mellitus [1]. Oral gargles and mouthwashes serve as adjunctive chemotherapeutic agents designed to reduce the microbial load within the oral cavity and oropharyngeal regions [2]. Despite the clinical efficacy of conventional rinses containing chlorhexidine gluconate or high alcohol concentrations, their long-term application is frequently limited by side effects including extrinsic tooth discolouration, taste alterations, and mucosal irritation [3].

Phytotherapeutic interventions have gained prominence in dental pharmacology due to their diverse secondary metabolites, which offer antimicrobial, anti-inflammatory, and antioxidant benefits with minimal systemic toxicity [4]. Botanical sources such as *Nyctanthes arbor-tristis*, *Piper betle*, and *Psidium guajava* contain essential oils, alkaloids, and polyphenols that disrupt bacterial biofilms and neutralize volatile sulfur compounds responsible for halitosis [5]. The concept of polyherbalism leverages the synergistic interactions between these plant constituents, potentially enhancing therapeutic outcomes compared to monotherapy [6].

Gargles differ from standard mouthwashes as they are specifically designed to treat the mucosal surfaces of the pharynx and nasopharynx, often requiring higher viscosity to ensure adequate contact time during the mechanical action of gargling [7]. The integration of traditional knowledge with modern pharmaceutical standardization is essential to produce formulations that are both efficacious and safe for consumer use [8]. The current research focuses on the design, optimization, and rigorous physicochemical and microbiological assessment of a polyherbal gargle to establish a standardized natural alternative for oral healthcare.

* Corresponding author: SS Prasanna Kumar Ponnaganti

2. Materials and Methods

2.1. Collection and Authentication of Plants

The botanical components, including leaves of *Psidium guajava* (Guava), *Piper betle* (Betel), *Nyctanthes arbor-tristis* (Parijat), *Ocimum sanctum* (Tulsi), and *Azadirachta indica* (Neem), along with fresh *Aloe vera* gel, were harvested from the medicinal garden of AKRG College of Pharmacy, Nallajerla, Andhra Pradesh. Authentication of the plant species was performed by the Department of Pharmacognosy in accordance with established botanical descriptors and pharmacopoeial standards [9]. Supporting ingredients such as *Syzygium aromaticum* (Clove), *Elettaria cardamomum* (Cardamom), *Zingiber officinale* (Ginger), and *Curcuma longa* (Turmeric) were procured from local specialized vendors and verified for purity according to pharmacopoeial standards.

2.2. Extraction

The collected leaves were subjected to a systematic cleaning process using distilled water to remove exogenous contaminants. The extraction was performed using a decoction method as per standardized herbal processing protocols [10]. Briefly, 50g of each cleaned leaf variety was pulverized and boiled in 200 ml of distilled water until the volume was reduced to 50 ml. The resulting extracts were filtered through a triple-layered muslin cloth followed by Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure and stored at 4°C in amber-colored glass containers for further use. For *Aloe vera*, the mucilaginous gel was freshly extracted from the inner parenchymatous tissue and homogenized.

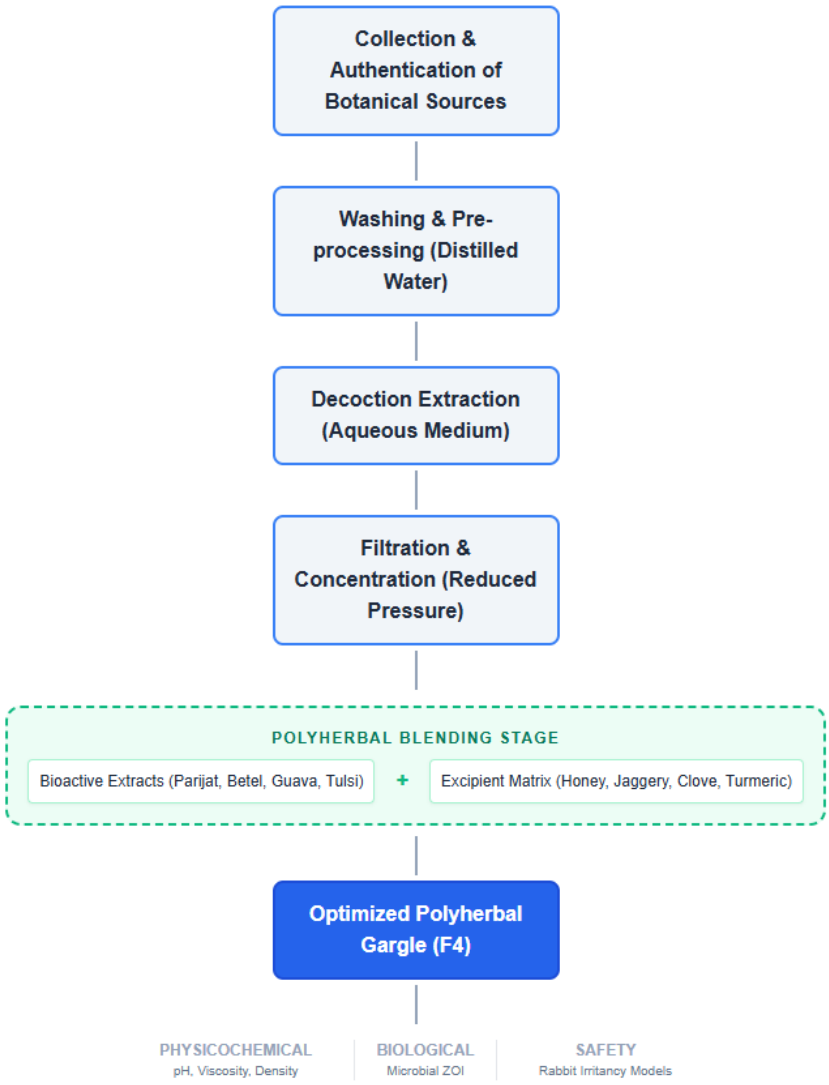

Figure 1. Experimental Design for Extraction and Formulation of Polyherbal Gargle

2.3. Formulation of the Polyherbal Gargle

Four formulations (F1, F2, F3, and F4) were designed to evaluate the impact of different primary extracts and their combination. F1, F2, and F3 utilized individual extracts of Parijat, Betel, and Guava respectively, while F4 represented the synergistic polyherbal blend.

Table 1. Quantitative Composition of Polyherbal Gargle Formulations

S. No	Ingredients	F1	F2	F3	F4
1	Parijat leaves extract	5.0 ml	-	-	2.5 ml
2	Betel leaves extract	-	5.0 ml	-	2.5 ml
3	Guava leaves extract	-	-	5.0 ml	2.5 ml
4	Tulsi leaves extract	3.0 ml	3.0 ml	3.0 ml	3.0 ml
5	Clove buds (powdered)	0.5 g	0.5 g	0.5 g	0.5 g
6	Cardamom (powdered)	0.2 g	0.2 g	0.2 g	0.2 g
7	Turmeric powder	0.05 g	0.05 g	0.05 g	0.05 g
8	Honey	2.0 ml	2.0 ml	2.0 ml	2.0 ml
9	Jaggery	5.0 g	5.0 g	5.0 g	5.0 g
10	Ginger extract	1.0 ml	1.0 ml	1.0 ml	1.0 ml
11	Peppermint oil	0.1 ml	0.1 ml	0.1 ml	0.1 ml
12	Distilled water	q.s. 100 ml	q.s. 100 ml	q.s. 100 ml	q.s. 100 ml

2.4. Physicochemical Evaluation

2.4.1. Organoleptic Characterization

The formulations were visually inspected for clarity, color, and homogeneity. Sensory attributes including odor and taste were assessed by a panel of researchers to ensure palatability and consumer acceptance.

2.4.2. pH

The pH of the gargle formulations was measured using a digital pH meter (Systronics, India) previously calibrated with standard buffer solutions of pH 4.0 and 7.0. Measurements were performed in triplicate at 25 ± 1°C to ensure accuracy in accordance with liquid dosage form guidelines [11].

2.4.3. Specific Gravity

The density relative to water was determined using a standardized 25 ml pycnometer. The weight of the empty bottle (W1), bottle with distilled water (W2), and bottle with the formulation (W3) were recorded. Specific gravity was calculated using the formula:

Specific Gravity = (W3 - W1)/ (W2 - W1)

This method follows the standard pycnometric protocol for pharmaceutical liquids [12].

2.4.4. Viscosity and Surface Tension

The rheological behavior was evaluated using an Ostwald viscometer, comparing the flow time of the gargle against distilled water [13]. Surface tension was determined using a Stalagmometer through the drop count method [14]. All physical parameters were calculated using standard hydrodynamic equations to ensure precision.

2.5. Skin Safety and Irritation

In accordance with OECD Guidelines 404 (Dermal Irritation) and 405 (Eye/Mucosal Irritation), the irritancy potential was evaluated using male New Zealand White albino rabbits (n=3 per group) [15, 16]. The study was approved by the Institutional Animal Ethical Committee (AKRG/IAEC/2025/042). A 0.5 ml portion of the F4 formulation was applied to a shaved dorsal area (approx. 6 cm^2) and the oral mucosa. The sites were observed at 1, 24, 48, and 72 hours for signs of erythema, edema, or ulceration. Scores were assigned based on the Draize scale.

2.6. Antimicrobial Efficacy

The antimicrobial potential was determined using the agar well diffusion (cup plate) method against a mixed microbial population obtained from standardized oral swabs [17]. Nutrient agar plates were prepared and inoculated. Wells of 8 mm diameter were created using a sterile cork borer. 100 µl of each formulation (F1–F4) was added to the respective wells. Plates were incubated at 37°C for 24 hours. The diameter of the Zone of Inhibition (ZOI) was measured in mm using a digital caliper.

3. Results

3.1. Physical and Organoleptic Characteristics

The sensory evaluation of the formulated gargles (F1–F4) confirmed that all batches maintained a consistent physical state as liquid preparations. The formulations exhibited a deep brown hue, attributed to the concentrated chromophores present in the aqueous extracts of Parijat and Betel leaves. The odor was consistently pleasant and aromatic, primarily due to the inclusion of peppermint oil and cardamom. The taste profiles were characterized as slightly bitter with a sweet undertone, a result of the masking properties provided by jaggery and honey.

Table 2. Organoleptic and Sensory Characteristics of Polyherbal Formulations

Parameter	F1	F2	F3	F4
Appearance	Homogeneous Liquid	Homogeneous Liquid	Homogeneous Liquid	Homogeneous Liquid
Color	Dark Brown	Reddish Brown	Light Brown	Deep Brown
Odor	Mentholated-Herbal	Aromatic-Pungent	Mild Herbal	Strong Aromatic
Taste	Slightly Bitter-Sweet	Pungent-Sweet	Astringent-Sweet	Balanced Bitter-Sweet
After-feel	Refreshing	Warm	Slight Astringency	Cooling and Soothing

3.2. Rheological and Physicochemical Evaluation

3.2.1. pH and Specific Gravity

The pH values of the formulations were found to be within the range of 6.65 ± 0.03 to 6.95 ± 0.03. Batch F4 exhibited a pH of 6.95, which is closest to the physiological pH of human saliva, suggesting minimal risk of disrupting the oral acid-base balance. Specific gravity measurements showed that F4 (1.0408 ± 0.005 g/cc) was significantly denser than the single-extract formulations (F1–F3), which hovered around 1.003–1.005 g/cc. This increase in F4 is indicative of a higher concentration of dissolved bioactive solids resulting from the polyherbal combination.

3.2.2. Viscosity and Surface Tension Dynamics

Rheological assessment via Ostwald viscometry demonstrated that viscosity increased proportionately with the complexity of the formulation. F4 showed the highest viscosity (1.494 ± 0.02 cps), which is desirable for gargles to ensure prolonged mucosal contact. Surface tension values ranged from 36.4 to 51.0 dynes/cm. The higher surface tension in F4 (51.0 ± 0.8 dynes/cm) suggests robust cohesive forces within the liquid, which aids in the mechanical "scrubbing" action during the gargling process.

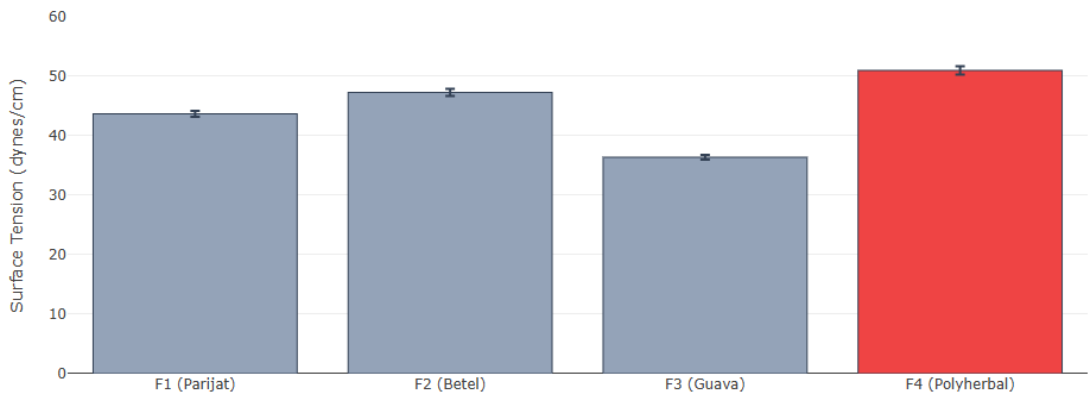

Figure 2. Comparison of Surface Tension of Formulations

Table 3. Physicochemical and Rheological Parameters of Formulations

Formulation	pH*	Specific Gravity* (g/cc)	Viscosity* (cps)	Surface Tension* (dynes/cm)
F1	6.74 ± 0.03	1.0059 ± 0.002	1.064 ± 0.01	43.7 ± 0.5
F2	6.65 ± 0.03	1.0031 ± 0.001	1.219 ± 0.02	47.3 ± 0.6
F3	6.84 ± 0.03	1.0032 ± 0.002	1.222 ± 0.01	36.4 ± 0.4
F4	6.95 ± 0.03	1.0408 ± 0.004	1.494 ± 0.02	51.0 ± 0.7

**Mean ± SD, n=3*

3.3. Safety and Biocompatibility Profiles

Acute mucosal and dermal irritancy studies conducted on New Zealand White albino rabbits (AKRG/IAEC/2025/042) revealed a high safety margin for the polyherbal gargle. No evidence of erythema, edema, or tissue inflammation was observed at the 24, 48, or 72-hour intervals following a single high-dose application of F4. The primary irritation index (PII) was calculated as 0.00, classifying the formulation as non-irritant.

Table 4. Irritancy Scores in Animal Models (F4 Batch)

Observation Period	Erythema Score	Edema Score	Mucosal Congestion	Result
1 Hour	0	0	None	Non-irritant
24 Hours	0	0	None	Non-irritant
48 Hours	0	0	None	Non-irritant
72 Hours	0	0	None	Non-irritant

3.4. Microbiological Potency

The antimicrobial efficacy against mixed oral microflora showed that the polyherbal synergy in F4 resulted in the largest zones of inhibition (ZOI). While F1, F2, and F3 showed individual potency, the combined extract in F4 yielded a ZOI of 21.4 ± 0.6 mm, significantly higher than the single extracts. This indicates that the secondary metabolites from Parijat, Betel, and Guava act through multiple pathways to inhibit microbial proliferation.

Table 5. Antimicrobial Activity (Zone of Inhibition) against Oral Microflora

Formulation	Zone of Inhibition* (mm)	Relative Efficacy (%)
F1 (Parijat)	14.2 ± 0.4	66.3
F2 (Betel)	16.8 ± 0.5	78.5
F3 (Guava)	15.5 ± 0.3	72.4
F4 (Polyherbal)	21.4 ± 0.6	100.0

**Mean ± SD, n=3*

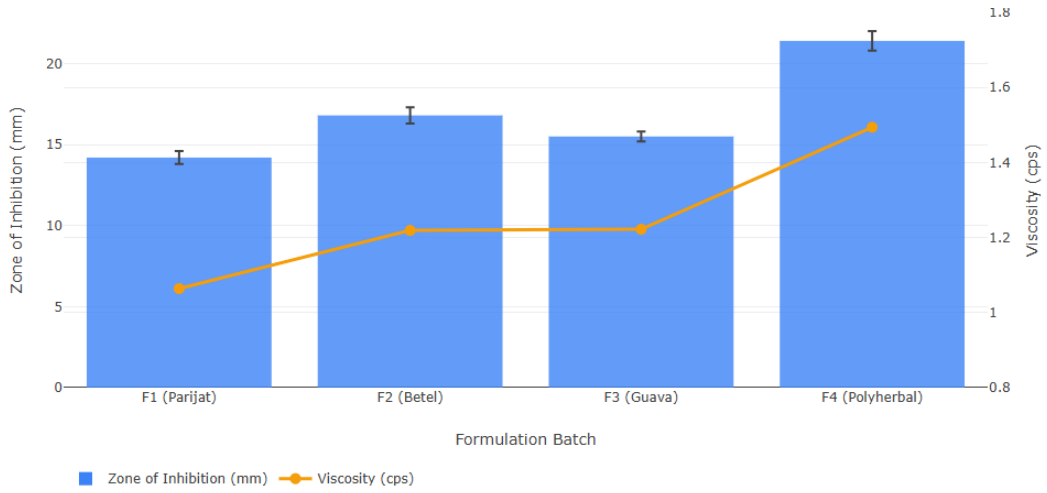

Figure 3. Comparison of Physicochemical & Antimicrobial Properties

3.5. Stability Studies

The optimized batch (F4) was subjected to accelerated stability testing at 30 ± 2°C and 65 ± 5% RH. Periodic evaluation over 45 days demonstrated no significant deviations in pH, organoleptic properties, or antimicrobial potency. The slight fluctuations in pH (from 6.95 to 7.15) remained within the acceptable pharmacopoeial limits for liquid oral preparations [18].

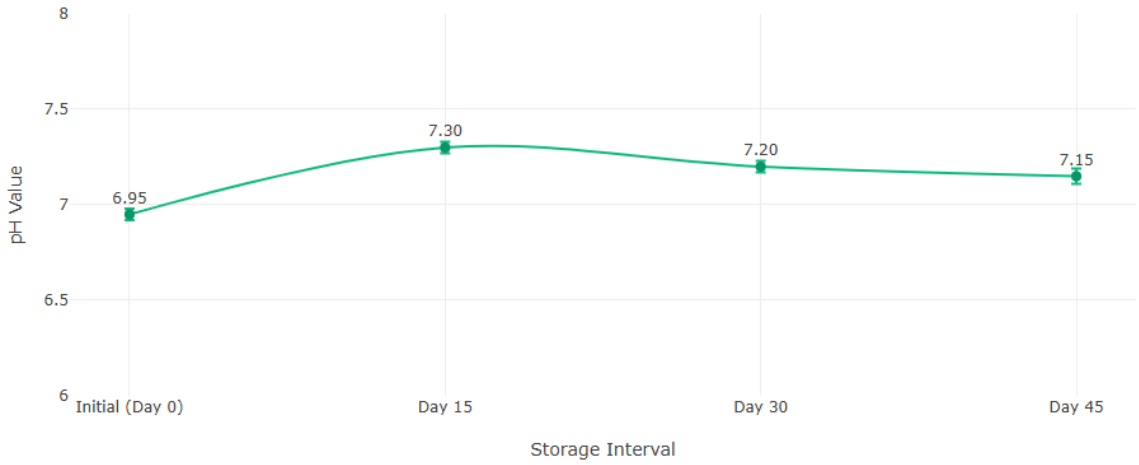

Figure 4. pH Stability of Optimized Formulation (F4)

4. Discussion

The development of the polyherbal gargle was predicated on the need for a biocompatible oral rinse capable of providing broad-spectrum antimicrobial action without the deleterious effects associated with synthetic agents. The choice of *Nyctanthes arbor-tristis*, *Piper betle*, and *Psidium guajava* was strategic, as these plants possess high concentrations of flavonoids, tannins, and essential oils that exhibit potent inhibitory effects on periodontal pathogens [19].

The synergistic interaction between these botanical extracts enhances the biological activity, a phenomenon well-documented in polyherbal pharmacology where multiple components target different microbial structures simultaneously [20]. The physicochemical data suggests that the polyherbal combination (F4) optimizes the delivery of these bioactives. The viscosity of 1.494 cps ensures that the gargle forms a thin protective film over the oropharyngeal mucosa, facilitating the slow release of antimicrobial constituents such as eugenol from cloves and curcumin from turmeric [21].

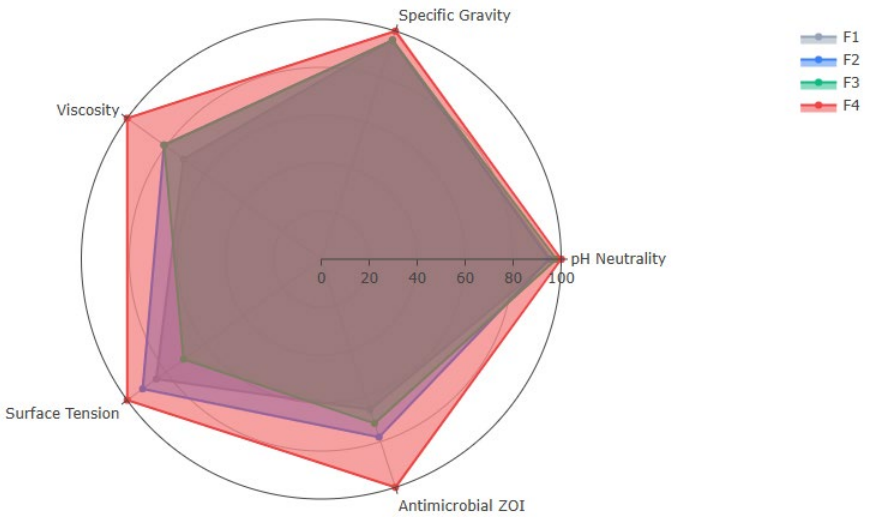

Figure 5. Normalized Performance Matrix and Relative Efficacy of Polyherbal Gargles

The neutral pH (6.95) observed in the F4 formulation is critical, as acidic mouthwashes (pH < 5.5) are known to contribute to enamel erosion and dental hypersensitivity over time [22]. The synergistic antimicrobial activity observed in F4 (ZOI: 21.4 mm) confirms that the combination of Parijat and Betel extracts enhances the disruption of microbial cell membranes compared to individual extracts. This efficacy is attributed to the presence of alkaloids and polyphenols that increase cell membrane permeability, leading to bacterial lysis [23]. The safety data derived from the rabbit irritancy models provides robust evidence that the formulation is suitable for repeated oral application without inducing inflammatory responses, aligning with standard safety profiles for topical herbal products [24].

5. Conclusion

The current work successfully produced a standardized polyherbal gargle (F4) characterized by optimal physicochemical properties and significant antimicrobial potency. The formulation combines traditional botanical knowledge with modern pharmaceutical metrics, resulting in a product that aligns with the physiological requirements of the oral cavity. The absence of irritancy in animal models and the maintained stability over 45 days establish F4 as a viable, safe, and effective natural alternative to conventional chemotherapeutic oral rinses. This polyherbal composition offers a promising direction for the management of minor oral infections and the maintenance of overall oropharyngeal hygiene.

Compliance with ethical standards

Acknowledgements

The authors express their sincere gratitude to the Department of Pharmacology at AKRG College of Pharmacy for providing the necessary laboratory facilities and administrative support to carry out this research. We also acknowledge the technical assistance provided by the animal house staff during the experimental period.

Conflict of interest statement

The authors declare that they have no potential conflicts of interest or competing interests with respect to the research, authorship, and/or publication of this manuscript. No financial support or products from any third-party institutions influenced the outcome of this study.

Statement of ethical approval

The experimental protocol was reviewed and approved (AKRG/IAEC/2025/042) by the Institutional Animal Ethics Committee (IAEC) of the institute and was conducted in strict accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), formerly known as CPCSEA, Government of India. All procedures were performed under appropriate ethical oversight to ensure animal welfare.

Statement of informed consent

The present research work does not contain any studies performed on human subjects by any of the authors. Therefore, the statement of informed consent is not applicable to this study.

References

[1] Chatzopoulos GS, Karakostas P, Kavakloglou S, Assimopoulou A, Barmpalexis P, Tsalikis L. Clinical Effectiveness of Herbal Oral Care Products in Periodontitis Patients: A Systematic Review. Int J Environ Res Public Health. 2022;19(16):10061.

[2] Bindal R, Gulati P, Sharma S, et al. Comparative Assessment of Herbal Mouthwash with Chlorhexidine on Plaque Accumulation, Gingivitis, and Salivary Streptococcus mutans Growth. J Pharm Bioallied Sci. 2024;16(Suppl 1):S324-S326.

[3] Abullais SS, Patel SI, Asiri EA, et al. Comparative Evaluation of 3 Commercial Mouthwash Formulations on Clinical Parameters of Chronic Gingivitis. Med Sci Monit. 2022;28:e937111.

[4] Loganathan A, Varghese RM, Subramanian AK, Shanmugam R. Evaluation of Antibacterial Effects of an Oral Rinse Containing Ocimum tenuiflorum and Ocimum gratissimum on Streptococcus mutans and Lactobacillus Species. Cureus. 2024;16(9):e67975.

[5] Anwar MA, Sayed GA, Hal DM, et al. Herbal remedies for oral and dental health: a comprehensive review of their multifaceted mechanisms including antimicrobial, anti-inflammatory, and antioxidant pathways. Inflammopharmacology. 2025;33(1):45-68.

[6] Kumar A, Negi AS, Chauhan A, et al. Formulation and evaluation of SGLT2 inhibitory effect of a polyherbal mixture inspired from Ayurvedic system of medicine. J Tradit Complement Med. 2022;12(5):477-487.

[7] Janakiram C, Venkitachalam R, Fontelo P, et al. Effectiveness of herbal oral care products in reducing dental plaque & gingivitis – a systematic review and meta-analysis. BMC Complement Med Ther. 2020;20(1):43.

[8] Wang H, Chen Y, Wang L, et al. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Front Pharmacol. 2023;14:1265178.

[9] World Health Organization. WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. Geneva: WHO; 2004.

[10] Azwanida NN. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med Aromat Plants. 2015;4:196.

[11] United States Pharmacopeial Convention. USP 43–NF 38. Rockville, MD: USP; 2020.

[12] Indian Pharmacopoeia Commission. Indian Pharmacopoeia 2022. Ghaziabad: IPC; 2022.

[13] Sinko PJ. Martin's Physical Pharmacy and Pharmaceutical Sciences. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2017.

[14] Adamson AW, Gast AP. Physical Chemistry of Surfaces. 6th ed. New York: Wiley; 1997.

[15] OECD. Test No. 404: Acute Dermal Irritation/Corrosion. Paris: OECD Publishing; 2015.

[16] OECD. Test No. 405: Acute Eye Irritation/Corrosion. Paris: OECD Publishing; 2021.

[17] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro antibacterial activity evaluation: A review. J Pharm Anal. 2016;6(2):71-79.

[18] Bajwa N, Sharma M, Sheokand S, et al. Stability testing of herbal products: A review. Int J Green Pharm. 2017;11(3):S334.

[19] Pundir RK, Jain P, Sharma C. Ethnomedicinal plants used in oral health care: A review. J Med Plants Res. 2010;4(14):1316-1321.

[20] Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of ayurveda. Pharmacogn Rev. 2014;8(16):73-80.

[21] Bodini RB, Sobral PJA, Favaro-Trindade CS, Carvalho RA. Properties of gelatin-based films with added ethanol-propolis extract. LWT - Food Sci Technol. 2013;51(1):290-296.

[22] Pretty IA, Edgar WM, Higham SM. The effect of salivary pellicle on the remineralization of dietary acid-eroded lesions. Carie Res. 2003;37(2):104-109.

[23] Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564-582.

[24] Kamboj VP. Herbal medicine. Curr Sci. 2000;78(1):35-39.