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Formulation and in Vitro Evaluation of a Spilanthes Acmella Herbal Mouthrinse against Streptococcus Mutans

Dr. G. Shobana[1a,1b*], Dr. M. Senthil[2], Dr. D S. Dinesh[3], Dr. Lakshmiganthan Mahalingam[4], Dr. M. Sakthi Balan[5]

[1a] Ph.D. Research Scholar, Indira Gandhi Institute of Dental Sciences, Sri Balaji Vidyapeeth (Deemed-to-be-University), Pondicherry

[1b] Reader, Department of Public Health Dentistry, Sri Venkateshwaraa Dental College, Puducherry

[2]Professor and Team Leader, Department of Public Health Dentistry, Indira Gandhi Institute of Dental Sciences, Sri Balaji Vidyapeeth (Deemed-to-be-University), Pondicherry

[3]Professor & Head, Department of Conservative Dentistry and Endodontics, Sri Venkateshwaraa Dental College, Puducherry

[4]Professor, Department of Periodontics, Karpaga Vinayaga Institute of Dental Sciences, Chengalpattu, Tamil Nadu

[5]Professor & Head, Department of Pharmacology, Sri Venkateshwaraa Medical College and Hospital Research Centre, Puducherry

Abstract:-This investigation aimed to develop a herbal mouthrinse incorporating an ethanolic extract of Spilanthes acmella and to characterise its physicochemical attributes alongside its in vitro inhibitory activity against Streptococcus mutans. A herbal mouthrinse was compounded using a standardised S. acmella ethanolic extract as the active component within a proprietary hydroalcoholic vehicle system incorporating pharmaceutical excipients. A comprehensive physicochemical evaluation was conducted covering pH, viscosity, surface tension, organoleptic characteristics, salivary compatibility, and microbiological safety. Inhibitory activity against S. mutans (MTCC, Chandigarh) was evaluated through disc diffusion assay at volumes of 50, 100, 150, and 200 µl, with 0.2% chlorhexidine gluconate as the reference standard. The mouthrinse displayed a pH of 6.0, a viscosity of 2.1 ± 0.1 cP, and a surface tension of 35.2 ± 1.5 dynes/cm. Organoleptic assessment yielded a clear, pale-green, uniform preparation with a refreshing menthol character and a patient acceptability rating of 4.5/5. Microbiological testing confirmed safety with a total viable count of 12 CFU/mL and no pathogenic growth. Disc diffusion assay revealed concentration-dependent inhibition against S. mutans, with zones of inhibition ranging from 2.75 ± 0.19 mm at 50 µl to 11.44 ± 0.80 mm at 200 µl, versus 14.72 ± 1.03 mm for the chlorhexidine control. The S. acmella herbal mouthrinse exhibited clinically acceptable physicochemical properties, adequate microbiological safety, and meaningful concentration-dependent inhibitory activity against S. mutans, supporting its potential as a plant-derived adjunct to conventional dental hygiene strategies.

Keywords: Spilanthes acmella, Spilanthol, Physiochemical evaluation.

1. Introduction

Oral caries is a complex, biofilm-associated, fermentable carbohydrate-dependent condition representing a substantial challenge to public health systems worldwide. Epidemiological data from the World Health Organization indicate that oral diseases collectively affect close to 3.5 billion individuals globally, with untreated decay in permanent teeth recognised as the most prevalent health condition documented worldwide [1]. Streptococcus mutans, a gram-positive facultatively anaerobic species that colonises the human oral cavity, is broadly acknowledged as the central initiating organism in cariogenesis, owing to its glucosyltransferase-mediated adhesion, biofilm formation, and lactic acid production from dietary sugars [2].

Chlorhexidine gluconate at 0.2% concentration remains the benchmark antimicrobial rinse in clinical dental practice. Notwithstanding its efficacy, sustained chlorhexidine use is frequently associated with tooth and tongue discolouration, impaired taste acuity, mucosal sensitivity, and supragingival calculus accumulation [3]. These shortcomings have motivated a sustained research effort directed at identifying botanical alternatives with comparable antibacterial potency and a more favourable tolerability profile.

Spilanthes acmella (L.) Murr., a tropical herbaceous plant of the Asteraceae family, has been employed in folk medicinal traditions throughout Asia and Africa for relief of toothache, control of oral infections, and management of inflammatory oral disorders [4, 5]. Its principal phytochemical constituent, spilanthol (N-isobutyl-2,6,8-decatrienamide), has been well documented to possess antibacterial, antifungal, anti-inflammatory, and immunomodulatory activities [6, 7, 8].

A body of in vitro experimental evidence has documented the activity of S. acmella preparations against prevalent oral microorganisms, including S. mutans, Lactobacillus acidophilus, and Candida albicans [2, 9, 10, 11]. Ismail et al. (2020) reported that a mouthrinse prepared from S. acmella produced a salivary S. mutans reduction in paediatric subjects comparable to that of the standard chlorhexidine preparation [2]. Despite encouraging biological evidence, systematic pharmaceutical development of an S. acmella mouthrinse inclusive of rigorous physicochemical characterisation and standardised antimicrobial testing remains insufficiently addressed in the literature.

2. Objectives

The current study was therefore designed to prepare a stable herbal mouthrinse from a standardised ethanolic extract of S. acmella and to undertake a thorough evaluation of its physicochemical attributes, including pH, viscosity, surface tension, organoleptic profile, and salivary compatibility, together with an in vitro antibacterial assessment against S. mutans using the disc diffusion methodology.

3. Methods

Plant Material and Extraction

This study was conducted at Sri Venkateswaraa Dental College and Hospital, Puducherry, during the academic year 2025–2026. Microbiological assays were performed at Veridian Micro Lab Pvt Ltd, Kelambakkam, Chennai, India. Freshly harvested leaves of Spilanthes acmella were procured, verified taxonomically, and subjected to shade-drying. The dried leaf material was ground to a uniform powder and subjected to ethanolic extraction under controlled temperature and time conditions. Following filtration, the resulting extract was concentrated by evaporation under reduced pressure and held at 4°C until further use. Specific extraction parameters, including solvent concentration, temperature, duration, and yield, constitute part of the proprietary formulation data withheld pending patent protection.

Mouthrinse Preparation

The mouthrinse formulation was developed using the S. acmella ethanolic extract as the active pharmaceutical component. The formulation was composed of the active extract combined with a hydroalcoholic vehicle system incorporating a co-solvent, a surfactant-emulsifier, a non-cariogenic sweetening agent, and a flavouring agent, all selected in accordance with established pharmaceutical principles for oral liquid preparations [12, 13]. The final preparation was brought to a defined volume using purified water, processed under controlled conditions to ensure homogeneity, filtered through appropriate filtration media, and dispensed into light-resistant amber-glass containers to minimise photodegradation [12, 13]. The precise qualitative and quantitative composition of the formulation, including excipient identities, grades, concentrations, and preparation parameters, constitutes proprietary intellectual property and has been withheld from this disclosure pending patent protection.

Physicochemical Evaluation

pH assessment: Using a calibrated electronic pH meter cross-validated with indicator strips, pH was recorded in triplicate and the mean was determined. An acceptable pH window of 6 to 7 was used as the criterion for oral mucosal compatibility and non-irritancy [1].

Viscosity assessment: A Brookfield-type rotational viscometer operated at 25°C was employed to measure viscosity. Triplicate determinations were taken at a constant spindle speed and results were recorded as mean ± SD in centipoise (cP), using distilled water as the reference [13].

Surface tension assessment: The drop-count stalagmometric technique was used to determine surface tension. A fixed volume of 1 mL each of the test sample and distilled water was dispensed and the corresponding drop counts were recorded. Surface tension was derived using the inverse proportionality: surface tension $\propto$ 1/number of drops, with values reported in dynes/cm [14].

Organoleptic and sensory assessment: The mouthrinse was assessed by trained evaluators for visual appearance, clarity, smell, taste, and overall impression. Each parameter was graded on a 1-to-5 scale and an aggregate patient acceptability score was determined.

Salivary compatibility testing: Artificial saliva was combined with the mouthrinse in a 1:1 proportion and the mixture was observed at predetermined intervals (0 min, 30 min, 1 h, and 24 h) for physical instability signs including turbidity, precipitation, or phase separation [15].

Microbiological safety testing: Sterility and microbial load assessments were conducted according to the protocols specified in USP <61> and <62> [16, 17]. Total aerobic bacterial count was established by the standard pour-plate method on Nutrient agar. Selective agars were used to screen for specific pathogenic organisms: Eosin Methylene Blue (EMB) agar for E. coli, Blood agar for S. aureus, and Potato Dextrose agar (PDA) for C. albicans. All culture plates were maintained at 37°C for 24 to 48 h, with results quantified in colony-forming units per millilitre (CFU/mL) [18].

In Vitro Antibacterial Assay

The antimicrobial activity of the prepared mouthrinse against a certified S. mutans strain (MTCC, Institute of Microbial Technology, Chandigarh, India) was assessed by the Kirby-Bauer disc diffusion method, following the guidelines published by the National Committee for Clinical Laboratory Standards (NCCLS, 1993) [19].

Culture medium preparation: Nutrient agar (HiMedia) was hydrated by suspending 28 g in 1000 mL of distilled water, brought to boil with stirring, then sterilised by autoclaving at 121°C (15 psi) for 15 min. The sterile medium was poured into Petri dishes under aseptic conditions and allowed to solidify.

Bacterial inoculum preparation: A loopful of the S. mutans strain was emulsified in physiological saline and sub-cultured onto Nutrient agar slants, followed by incubation at 37°C for 24 h. The resulting cultures were stored at 2–8°C for subsequent experiments.

Disc diffusion procedure: Sample volumes of 50, 100, 150, and 200 µl were prepared for testing. Sterile Whatman No. 1 filter paper discs (6 mm diameter) were individually impregnated with the corresponding volumes and stored refrigerated at 4°C for 24 h. A standardised bacterial suspension was uniformly spread onto Nutrient agar plates using sterile cotton swabs, and the impregnated discs were aseptically placed using forceps with full surface contact ensured. Plates were pre-cooled at 5°C for 1 h to facilitate uniform disc diffusion before transfer to a 37°C incubator for 24 h. Chlorhexidine gluconate 0.2% (30 µl) served as the positive reference control, and all assays were conducted in triplicate [19, 20]. The diameter of the inhibition zone around each disc was measured with a millimetre-calibrated scale and reported as mean ± SD.

Statistical Analysis

All quantitative measurements were performed in triplicate and expressed as mean ± standard deviation (SD). Data were analysed using IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were applied to physicochemical parameters. For the antibacterial assay, a one-way analysis of variance (ANOVA) was used to compare zones of inhibition across the four test volumes and the chlorhexidine reference, followed by Tukey's post hoc test for pairwise comparisons. A p-value of less than 0.05 was considered statistically significant.

4. Results

Physicochemical Evaluation

The prepared herbal mouthrinse presented as a clear, pale-green, homogeneous liquid with no visible sediment or turbidity. The formulation recorded a mean pH of 6.0, falling within the 6 to 7 range considered suitable for oral preparations. A mean viscosity of 2.1 ± 0.1 cP at 25°C was recorded relative to 1.0 ± 0.0 cP for the distilled water reference, a marginally elevated value anticipated to facilitate improved retention and contact duration on oral surfaces. Detailed viscosity data are summarised in Table 1 ($p<0.05$ versus distilled water).

Table 1: Viscosity measurements of the formulated herbal mouthrinse at 25°C

Sample	Temp (°C)	Rep 1 (cP)	Rep 2 (cP)	Mean ± SD (cP)
Mouthwash (Test)	25	2.1	2.0 / 2.2	2.1 ± 0.1
Distilled water (Standard)	25	1.0	1.0 / 1.0	1.0 ± 0.0

The mouthrinse registered a surface tension of 35.2 ± 1.5 dynes/cm (95 drops/mL), markedly lower than the distilled water reference of 72 dynes/cm (50 drops/mL), as summarised in Table 2.

Table 2: Surface tension measurements using the stalagmometric drop-count technique

Sample	No. of Drops	Volume (mL)	Surface Tension (dynes/cm)	Interpretation
Distilled water	50	1	72.0	Standard reference
Mouthwash (Test)	95	1	35.2 ± 1.5	Enhanced spreading

The product was visually clear, pale green, and uniform, exhibiting a pleasant cooling menthol aroma, a mildly sweet taste, and a patient acceptability score of 4.5 out of 5. Physical stability was maintained throughout the salivary compatibility observation period, with no precipitation or phase separation detected at 0, 30, or 24 hours. A transient mild haziness noted at the 1-hour mark resolved completely by 24 hours and was not considered clinically significant. The complete physicochemical characterisation dataset is presented in Table 3.

Table 3: Consolidated physicochemical profile of the herbal mouthrinse

Parameter	Observed Result	Acceptable Range / Interpretation
pH	6.0	6–7; non-irritant, mucosally safe
Viscosity (cP)	2.1 ± 0.1	Marginally above water; optimal flow and mucosal retention
Surface tension (dynes/cm)	35.2 ± 1.5	Well below water (72); improved wetting and spreading capacity
Organoleptic appearance	Pale green, clear, homogeneous	Satisfactory

Odour / Taste	Menthol / Mildly sweet	Satisfactory
Patient acceptability	4.5 / 5	Highly acceptable
Salivary compatibility (1 h)	Slight transient haziness; no separation	Fully compatible with saliva

Microbiological Safety Evaluation

The total viable bacterial count was 12 CFU/mL, comfortably within the USP stipulated upper threshold of <100 CFU/mL for non-sterile liquid oral preparations [16, 17]. Targeted pathogen screening yielded negative results for E. coli, S. aureus, and C. albicans across all selective media employed, as recorded in Table 4.

Table 4: Microbiological safety profile of the formulated herbal mouthrinse

Assessment	Target Organism	Method	Result	Outcome
Total viable count	Total aerobic bacteria	Plate count (Nutrient agar)	12 CFU/mL	Within limit
Pathogen screen	Escherichia coli	EMB agar	Not detected	Safe
Pathogen screen	Staphylococcus aureus	Blood agar	Not detected	Safe
Pathogen screen	Candida albicans	PDA	Not detected	Safe

In Vitro Antibacterial Activity Against Streptococcus mutans

The mouthrinse exhibited a clear, volume-dependent antibacterial response against S. mutans. Inhibition zones expanded progressively from 2.75 ± 0.19 mm at 50 µl to 11.44 ± 0.80 mm at 200 µl. The chlorhexidine reference at 0.2% concentration generated an inhibition zone of 14.72 ± 1.03 mm. One-way ANOVA demonstrated a statistically significant difference in inhibition zone diameters across all test groups ($p<0.001$). Post hoc analysis (Tukey's test) confirmed that each successive volume increment produced a significantly larger zone of inhibition compared with the preceding concentration ($p<0.05$). Full data are presented in Table 5.

Table 5: Inhibitory activity of the S. acmella herbal mouthrinse against S. mutans (disc diffusion)

Test Volume / Concentration	Zone of Inhibition (mm), Mean ± SD
50 µl	2.75 ± 0.19
100 µl	4.26 ± 0.29
150 µl	8.51 ± 0.59
200 µl	11.44 ± 0.80
Chlorhexidine 0.2% (reference)	14.72 ± 1.03

Values presented as Mean ± SD (n = 3).

5. Discussion

This investigation successfully achieved the development and systematic characterisation of a novel plant-derived mouthrinse based on an ethanolic extract of Spilanthes acmella, and established its in vitro inhibitory efficacy against Streptococcus mutans in a concentration-responsive manner.

The rationale for selecting S. acmella as the active botanical ingredient rested on its well-established traditional applications in oral medicine and the accumulated scientific evidence supporting the inhibitory action of spilanthol against oral bacterial species [4, 5, 6, 7]. The alkylamide constituents in this plant are understood to compromise bacterial membrane integrity and attenuate the biofilm-forming capacity of S. mutans, both central to its virulence [10, 11].

The selection of excipients was guided by established pharmaceutical formulation principles for oral liquid preparations [12, 13]. The vehicle system was designed to solubilise the lipophilic phytoconstituents of the extract, maintain physicochemical stability, and preserve antimicrobial integrity throughout the intended shelf life. Excipients were selected to confer appropriate rheological behaviour, surface activity, palatability, and patient acceptability while ensuring compatibility with the active botanical component. The specific identities and concentrations of excipients constitute proprietary formulation information and are withheld from this publication pending patent protection.

A formulation pH of 6.0, falling within the 6 to 7 range appropriate for oral use, ensures minimal potential for mucosal irritation, avoids erosive damage to hard tooth surfaces, and does not perturb the natural ecological balance of the oral microbiome [1]. The viscosity of 2.1 ± 0.1 cP, marginally exceeding that of water, is anticipated to extend the residence time of the active constituents on mucosal and dental surfaces, thereby optimising antimicrobial contact efficacy [13].

The pronounced reduction in surface tension from 72 dynes/cm (water) to 35.2 ± 1.5 dynes/cm reflects a meaningful enhancement in the wetting and spreading characteristics of the rinse. A lower surface tension promotes penetration into interproximal regions, crevicular sulci, and the structural matrix of biofilms — the primary ecological niches occupied by S. mutans — thereby augmenting the functional reach of the formulation [14]. Salivary compatibility testing confirmed physical integrity under oral conditions, with no clinically meaningful instability observed [15].

The microbiological safety assessment demonstrated full compliance with regulatory standards, registering a total viable count of 12 CFU/mL and complete absence of tested pathogenic species, satisfying the criteria laid out in USP <61> and <62> [16, 17] as well as relevant national pharmacopoeial guidelines [18].

The in vitro antibacterial evaluation revealed a statistically progressive, concentration-dependent reduction in S. mutans viability, with inhibition zones increasing from 2.75 ± 0.19 mm at 50 µl to 11.44 ± 0.80 mm at 200 µl. This pattern aligns with the published reports of Borate and Disale (2013) and Thompson et al. (2012), who both documented notable inhibitory activity of Acmella oleracea preparations against relevant dental microorganisms [10, 11].

When contrasted with the reference standard chlorhexidine gluconate 0.2% (zone of inhibition: 14.72 ± 1.03 mm), the mouthrinse at its highest tested volume (200 µl) achieved approximately 77.7% of the reference inhibitory effect. This relative performance is congruent with the clinical outcomes reported by Ismail et al. (2020), who found broadly comparable reductions in salivary S. mutans loads between an S. acmella mouthrinse and standard chlorhexidine in a paediatric clinical trial [2]. Additional corroboration was provided by Sathyaprasad et al. (2015), who reported significant inhibition of endodontic pathogens including Enterococcus faecalis and C. albicans [6], and by Jyotsna Srinath and Lakshmi (2014), who reviewed the broader dental therapeutic utility of the species [7].

The present study has several acknowledged limitations. Antibacterial efficacy was assessed using a single gram-positive cariogenic species. Subsequent investigations should include a wider microbial panel encompassing Lactobacillus acidophilus, Porphyromonas gingivalis, and Streptococcus sobrinus. Controlled clinical trials are needed to validate translational relevance. Furthermore, long-term physicochemical

stability data and formal minimum inhibitory concentration (MIC) determinations would substantially strengthen the pharmaceutical characterisation of this product.

6. Conclusion

A herbal mouthrinse formulated with an ethanolic extract of Spilanthes acmella met all targeted physicochemical criteria, demonstrating a pH of 6.0, a viscosity of 2.1 ± 0.1 cP, and a surface tension of 35.2 ± 1.5 dynes/cm, alongside satisfactory microbiological safety and high patient acceptability. In vitro antibacterial testing validated a concentration-responsive inhibitory action against Streptococcus mutans, with the highest tested concentration achieving approximately 77.7% of the inhibitory potency of 0.2% chlorhexidine. These findings collectively establish S. acmella as a scientifically credible active ingredient for a pharmaceutical-grade herbal mouthrinse. Clinical validation studies and extended stability investigations are recommended to support progression of this formulation toward clinical application as a natural, efficacious, and patient-friendly complement to established preventive dental care.

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Ethics committee approval

This study was carried out as a component of a doctoral research project and involved exclusively in vitro laboratory procedures. Institutional ethical clearance was granted by the relevant committee (SVDC/IEC-Cer/2024-25/99).

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