Development of a Herbal Mouthwash containing *Mimosa pudica* and Casein for Tannin Reduction

Miss. Pranjali S. Suryawanshi[1], Miss. Saundarya D. Rajput[2], Miss. Diksha Narwade[3]
[1,2]*Student, Sayali Charitable Trust's College of Pharmacy*
[3]*Assistant Professor, Sayali Charitable Trust's College of Pharmacy*

Abstract: Herbal mouthwashes are increasingly being recognized as safe and effective alternatives to conventional chemical-based oral care products due to their biocompatibility, minimal side effects, and natural therapeutic potential. This review focuses on the development of a novel herbal mouthwash containing *Mimosa pudica* and casein aimed at reducing tannin-induced staining and promoting overall oral health. *Mimosa pudica*, widely known for its antimicrobial, antioxidant, and anti-inflammatory properties, can help control oral microbial growth and oxidative stress, while casein, a milk-derived phosphoprotein, exhibits a high affinity for polyphenols and tannins, facilitating their precipitation and removal from the oral cavity. The formulation process typically involves the aqueous extraction of *Mimosa pudica* leaves using maceration or Soxhlet techniques, followed by filtration and concentration of the extract. Casein is then incorporated into the extract along with excipients such as humectants, preservatives, and flavoring agents to achieve a stable, palatable, and safe mouthwash. The final product is subjected to rigorous evaluation, including physicochemical parameters such as pH, viscosity, and organoleptic characteristics (taste, color, and odor), as well as stability studies under different storage conditions. Functional assessment involves measuring tannin-binding efficacy, antimicrobial activity against common oral pathogens, and cytotoxicity assays to ensure biocompatibility. The integration of plant bioactives with protein-based agents like casein provides a synergistic approach to reduce tannin levels, enhance oral hygiene, and offer a natural alternative to chemical mouthwashes. This review highlights the formulation strategies, mechanisms of tannin reduction, and evaluation parameters critical for developing effective herbal oral care products, demonstrating the potential of *Mimosa pudica* and casein-based mouthwashes as safe, natural, and functional oral hygiene solutions.

Keywords: *Mimosa pudica*, casein, herbal mouthwash, tannin reduction, oral health, formulation, natural oral care.

I. INTRODUCTION

Oral health is a vital component of general well-being, encompassing the prevention and management of dental caries, periodontal diseases, oral infections, and staining. Among the various factors affecting oral hygiene, tannins—polyphenolic compounds present in tea, coffee, certain fruits, and plant-derived foods—play a significant role in the discoloration of teeth and the formation of dental plaques. Tannins have a strong affinity for proteins in the saliva and the enamel pellicle, leading to precipitation, reduced oral cleanliness, and aesthetic concerns. Persistent exposure to tannin-rich diets can exacerbate these issues, contributing to enamel wear, altered oral pH, and microbial colonization. Conventional chemical mouthwashes, although effective in controlling microbial growth, often exhibit side effects such as altered taste perception, mucosal irritation, and long-term dependency. This has led to growing interest in natural and herbal alternatives that not only maintain oral hygiene but also reduce tannin-induced complications. Herbal formulations combine bioactive phytochemicals with biocompatible ingredients to offer preventive, therapeutic, and cosmetic benefits, making them a promising approach for modern oral care.

Herbal mouthwashes are liquid formulations derived from plant extracts, traditionally used in various cultures for maintaining oral hygiene. Unlike synthetic formulations, herbal mouthwashes are rich in phytochemicals, including flavonoids, tannins, alkaloids, and saponins, which exhibit antimicrobial, antioxidant, anti-inflammatory, and anticariogenic properties. Their natural origin ensures biocompatibility, minimal side effects, and long-term safety. Moreover, they are gaining popularity due to the increasing demand for natural oral care products that address both aesthetic and functional concerns. The mechanism of action of herbal mouthwashes often involves inhibition of bacterial adhesion, neutralization of free radicals, and reduction of enamel staining, providing a holistic approach to oral health. Incorporating protein-based agents like casein into herbal formulations enhances their functional properties, particularly in tannin binding and removal, thereby preventing discoloration and protecting dental surfaces. This integration of herbal bioactives and natural proteins represents a synergistic strategy for developing innovative oral hygiene solutions.

Mimosa pudica: Phytochemical and Therapeutic Relevance

Mimosa pudica, commonly known as the "sensitive plant", has been extensively studied for its medicinal and pharmacological properties. The plant is rich in alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which contribute to its antimicrobial, antioxidant, anti-inflammatory, and wound-healing activities. In oral care, *Mimosa pudica* extract can inhibit the growth of oral pathogens, reduce oxidative stress in the oral cavity, and support gum and enamel health. Its bioactive compounds can also complement protein-based agents such as casein to enhance tannin precipitation, thereby mitigating staining and astringency associated with tannin-rich diets. Studies have highlighted the potential of *Mimosa pudica* in herbal formulations, especially in synergistic combinations with other natural agents, making it an ideal candidate for mouthwash development. Furthermore, its aqueous extracts are water-soluble, stable, and compatible with common excipients, facilitating their incorporation into consumer-friendly oral care products.

Mimosa pudica, commonly known as the sensitive plant or touch-me-not, belongs to the Fabaceae family. It is a creeping perennial herb with characteristic bipinnate leaves that fold upon touch and pinkish spherical flowers. It is widely distributed in tropical and subtropical regions, including Asia, Africa, and South America. Traditionally, *Mimosa pudica* has been used in Ayurvedic and folk medicine for a variety of ailments such as wounds, inflammation, diarrhea, urinary disorders, and gynecological issues. Its leaves, roots, and stems are known for hemostatic, analgesic, anti-inflammatory, and antimicrobial properties, making it a versatile medicinal plant.

Phyto Chemical Composition:

Mimosa pudica contains a rich spectrum of bioactive compounds, each contributing to its pharmacological activities:

- Alkaloids – contribute to antimicrobial, analgesic, and neuroprotective effects.
- Flavonoids – potent antioxidants, scavenging free radicals and reducing oxidative stress in tissues.
- Tannins – exhibit astringent and antimicrobial activities.
- Saponins – enhance antimicrobial and anti-inflammatory effects.
- Phenolic compounds – provide antioxidant and anti-inflammatory properties.
- Glycosides and Terpenoids – show wound-healing and antimicrobial benefits.

These phytochemicals are responsible for the plant's therapeutic versatility, particularly in preventing microbial colonization, oxidative damage, and inflammation in the oral cavity and these properties make *Mimosa pudica* an ideal candidate for herbal mouthwash formulations, especially when combined with tannin-binding agents like casein.

Casein: Mechanism in Tannin Reduction

Casein is a milk-derived phosphoprotein widely recognized for its ability to interact with polyphenolic compounds. It binds tannins through hydrogen bonding and hydrophobic interactions, forming insoluble complexes that are easily removed from surfaces such as enamel and oral mucosa. This property not only helps in reducing staining but also contributes to oral protective effects by neutralizing the adverse effects of tannins, such as protein precipitation and reduced digestibility. Casein is also biocompatible, non-toxic, and cost-effective, making it a suitable additive for oral care formulations. When combined with herbal extracts like *Mimosa pudica*, casein can enhance the functional efficacy of the mouthwash by offering a dual action: microbial inhibition from the plant extract and tannin binding from the protein. This synergistic mechanism ensures improved oral aesthetics, hygiene, and overall health, representing a novel approach in herbal mouthwash development.

Casein is a major milk protein (~80% of bovine milk proteins) and exists in four main types: αs1-casein, αs2-casein, β-casein, and κ-casein. These phosphoproteins form micelles, which are stable aggregates capable of interacting with polyphenols, tannins, and metal ions. The protein's amphiphilic nature enables it to bind both hydrophilic and hydrophobic molecules, making it particularly useful in oral care applications. Tannins are polyphenolic compounds with a high propensity to bind proteins through hydrogen bonding and hydrophobic interactions. Casein binds tannins efficiently, forming insoluble complexes that can be easily removed from the teeth and oral surfaces.

Rationale for Developing a Mimosa pudica-Casein Mouthwash

The increasing consumer demand for natural, safe, and multifunctional oral care products highlights the need for formulations that address both microbial control and aesthetic concerns. A mouthwash combining *Mimosa pudica* and casein offers a holistic solution: the plant extract provides antimicrobial and antioxidant effects, while casein ensures effective tannin reduction. This formulation targets a common oral problem—tannin-induced staining—without relying on harsh chemicals. Furthermore, such mouthwashes can be easily standardized, evaluated for physicochemical properties, and tested for safety, making them suitable for commercialization. The integration of herbal bioactives with protein-based agents represents a promising strategy in oral care innovation, contributing to preventive dentistry and overall oral health. This approach also aligns with the global trend toward eco-friendly, sustainable, and natural healthcare products, providing an alternative to conventional chemical-based mouthwashes.

Objectives:

- To explore the phytochemical properties and therapeutic potential of *Mimosa pudica*, with special emphasis on its antimicrobial, antioxidant, and anti-inflammatory activities relevant to oral health.
- To investigate the role of casein in binding and reducing tannins, thereby preventing tannin-induced staining and enhancing oral aesthetics.
- To review formulation strategies for combining herbal extracts with protein-based agents in the development of a stable, palatable, and effective herbal mouthwash.
- To analyze the physicochemical and organoleptic evaluation parameters (such as pH, viscosity, color, taste, and stability) that ensure the quality and acceptability of herbal mouthwashes.
- To evaluate the functional efficacy of the mouthwash, including tannin-binding ability, antimicrobial activity against oral pathogens, and biocompatibility.
- To provide insights into the potential advantages of herbal-protein synergistic mouthwashes as safe, natural, and sustainable alternatives to conventional chemical-based oral care products.
- To identify research gaps and future perspectives for the development and commercialization of herbal mouthwashes targeting tannin reduction and overall oral health.

II. LITERATURE REVIEW AND DATA COLLECTION

1. Evaluation of Directly Compressible Cellulose Derived from Cassava Waste

Eraga et al. evaluated the direct compression suitability of microcrystalline cellulose (MCC) obtained from cassava fermentation waste in the formulation of Paracetamol tablets. The MCC produced locally demonstrated excellent flowability, low moisture content, and good compressibility, comparable to commercially available MCC (Avicel PH 102). Tablets prepared using this excipient showed acceptable hardness (4–5 kg/cm²), low friability (<1%), and fast disintegration (<5 min).

Relevance: The study confirms the feasibility of direct compression for Paracetamol using eco-friendly and cost-effective excipients, reinforcing that properly engineered cellulose derivatives can maintain tablet integrity and rapid drug release.

2. Formulation and Evaluation of Paracetamol Tablets Using Natural Binders

Kulkarni and co-researchers formulated Paracetamol immediate release tablets using natural binders such as coconut oil and compared them with conventional binders (e.g., PVP K-30). The results demonstrated that natural binders provided adequate tablet hardness and content uniformity, while maintaining a disintegration time of under 5 minutes. Dissolution studies showed that more than 90% of Paracetamol was released within 25 minutes, confirming immediate release characteristics.

Relevance: This study highlights the role of binder optimization in balancing tablet strength with rapid drug release, directly aligning with the objectives of developing an efficient Paracetamol IP 75 mg immediate release tablet.

3. Impact of Material Properties on wet granulation of Tablet Quality

A 2023 study reported on the impact of material properties and process parameters on tablet quality during continuous direct compression manufacturing. Parameters such as particle size distribution, moisture content, and lubrication level significantly affected tablet weight variation, hardness, and dissolution performance. The study emphasized the importance of excipient functionality and powder rheology in maintaining batch consistency.

Relevance: These findings are vital to understanding the critical quality attributes (CQAs) and critical process parameters (CPPs) that influence Paracetamol tablet quality in the direct compression process. It underscores the need for quality-by-design (QbD) approaches in formulation development.

III. METHODOLOGY / MATERIALS AND METHODS

Material

The herbal mouthwash formulation will utilize the following ingredients:

- Mimosa pudica leaf extract – provides antimicrobial, antioxidant, and anti-inflammatory activity.
- Casein – binds tannins, reduces staining, and protects enamel.
- Ocimum sanctum (Tulsi) extract – enhances antimicrobial and anti-inflammatory properties.
- Mentha piperita (Peppermint) oil – imparts flavor and mild antimicrobial effect.
- Syzygium aromaticum (Clove) oil – provides analgesic, antimicrobial, and antioxidant effects.
- Citrus limon (Lemon) juice/extract – provides astringent, refreshing properties and mild antibacterial activity.
- Aloe vera gel extract – provides soothing, anti-inflammatory, and wound-healing properties.
- Vegetable glycerin – serves as a humectant and solvent for flavoring agents.
- Honey – natural sweetener, antimicrobial, and humectant.
- Distilled water – used as the main aqueous base for extraction and formulation.

Equipments & Instruments:

- Analytical balance – for accurate measurement of plant materials, casein, and other ingredients.
- Mortar and pestle or grinder – to grind dried *Mimosa pudica* leaves and other herbs into fine powder.
- Soxhlet extractor or maceration setup – for aqueous extraction of herbal powders.
- Beakers and conical flasks – for soaking, mixing, and extraction of plant materials.
- Magnetic stirrer with hot plate – to maintain uniform mixing and mild heating during extraction.
- Whatman filter paper or muslin cloth – for filtration of extracts to remove insoluble residues.
- Rotary evaporator – to concentrate herbal extracts under reduced pressure (optional, but recommended for large-scale preparation).

Methodology

A. Preparation of *Mimosa pudica* Leaf Extract:

- Collect fresh leaves of *Mimosa pudica*, wash thoroughly to remove dust and impurities.
- Dry the leaves under shade at room temperature to preserve phytochemicals.
- Grind the dried leaves into a fine powder.
- Prepare an aqueous extract by maceration or Soxhlet extraction:
- Soak 50 g of powdered leaves in 500 mL distilled water for 24 hours with occasional stirring (maceration).
- Filter the extract using Whatman filter paper to remove solid residues.
- Concentrate the filtrate using a rotary evaporator or by gentle heating at 40–50°C until a viscous extract is obtained.

Purpose: This extract provides the primary antimicrobial and antioxidant activity for the mouthwash.

B. Preparation of Casein Solution:

- Dissolve the required amount of casein in a minimal volume of warm distilled water (40–50°C) with continuous stirring to ensure uniform dispersion.
- Adjust the pH to slightly acidic/neutral (6.5–7.0) to maintain protein stability.

Purpose: Casein will bind tannins and prevent enamel staining while stabilizing the formulation.

Batch 1 (Concentrations):

Mimosa pudica	Casein
3.5 ml	2 ml
3.5 ml	4 ml
3.5 ml	5 ml
3.5 ml	7 ml
3.5 ml	9 ml

Batch 2 (Concentrations):

Mimosa pudica	Casein
2 ml	2.5 ml
3 ml	3.5 ml
4.5 ml	4.5 ml
5 ml	5.5 ml
3.5 ml	5 ml

Incorporation of Additional Herbal Extracts:

- Prepare aqueous extracts of Ocimum sanctum and Aloe vera gel in the same manner as *Mimosa pudica*, or use standardized commercial extracts.
- Mix these extracts with the *Mimosa pudica* extract to form a homogeneous aqueous base.

Purpose: These extracts enhance antimicrobial, anti-inflammatory, and soothing properties.

Addition of Oils and Flavoring Agents:

- Add Mentha piperita oil (peppermint) and Syzygium aromaticum oil (clove) in small quantities (0.1–0.5% v/v each) to the extract mixture.
- Use vegetable glycerin as a solubilizing agent to disperse essential oils uniformly.
- Mix thoroughly using a magnetic stirrer to ensure proper emulsification.

Purpose: Provides flavor, aroma, and additional antimicrobial properties.

Addition of Citrus Extract and Honey:

- Add Citrus limon juice/extract in small quantities (1–2% v/v) to improve freshness and provide mild astringent and antibacterial effects.
- Add honey (1–2% w/v) to improve palatability and provide natural antimicrobial properties.
- Mix gently to avoid frothing.

Purpose: Improves taste, enhances antimicrobial activity, and acts as a natural humectant.

Final Mixing and Filtration:

- Combine the casein solution with the herbal-aqueous mixture slowly while stirring to avoid precipitation.
- Adjust the final volume with distilled water.
- Filter the final mixture through sterile muslin cloth or Whatman filter paper to remove insoluble particles.

Purpose: Ensures uniformity and stability of the mouthwash.

Packaging and Storage

- Transfer the mouthwash into sterile, airtight amber glass bottles to prevent degradation of phytochemicals and oils.
- Store at room temperature or 4°C to maintain stability and shelf-life.

C. Evaluation of Mouthwash:

Organoleptic Evaluation

1. Colour:
- Procedure: Observe the mouthwash against a white background under normal light.
- Evaluation: Check for uniformity, clarity, and whether the colour is acceptable and stable over time.
2. Odour:
- Procedure: Smell the mouthwash carefully at room temperature.
- Evaluation: Ensure there is no foul or unusual odour. The odour should be pleasant and consistent with the added flavoring.
3. Taste
- Procedure: (If safe) A small quantity can be tasted. Alternatively, a trained sensory panel can evaluate it.
- Evaluation: Assess the mouthwash for sweetness, bitterness, or pungency. Taste should be acceptable and masking of any herbal bitterness should be checked.
4. Appearance / Clarity:
- Procedure: Observe the solution for clarity, transparency, and presence of suspended particles.
- Evaluation: Mouthwash should be free from turbidity or precipitation.

Physical and Chemical Evaluation

1. pH Measurement

- Importance: Ensures the formulation is safe for oral mucosa (ideally 6.0–7.5) and prevents microbial growth.
- Procedure:
  i. Calibrate pH meter using standard buffer solutions (pH 4 and 7).
  ii. Measure the pH of the mouthwash at room temperature.
- Evaluation: Record the pH. Stability studies may require checking pH over time.

2. Viscosity:

- Importance: Ensures good mouthfeel and retention in the oral cavity.
- Procedure:
  i. Use a viscometer (Brookfield or similar).
  ii. Measure viscosity at a controlled temperature (usually 25°C).
- Evaluation: Compare with acceptable range (typically 1–50 cP for liquid mouthwashes).

Other Important Evaluation Parameters

1. Surface Tension:

- Importance: Affects spreading in the oral cavity.
- Procedure: Use a tensiometer to measure surface tension.

2. Foaming Capacity:

- Importance: Some mouthwashes are expected to foam slightly.
- Procedure: Shake a measured quantity and measure foam volume.

3. Microbial Load / Antimicrobial Activity:

- Importance: Ensures safety and efficacy against oral pathogens.
- Procedure:
  i. Plate count for total bacterial/fungal load by Agar Diffusion Method.
  ii. Test inhibition against common oral bacteria (e.g., *Streptococcus mutans*).

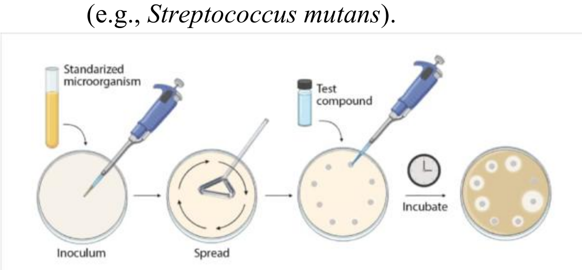

Fig. 1: Agar Diffusion Method

4. Stability Studies:

- Importance: To check long-term quality.
- Procedure:
  i. Store at different conditions (room temp, 40°C, refrigeration).
  ii. Evaluate periodically for pH, colour, odour, precipitation, and microbial growth.

5. Specific Gravity:

- Importance: Confirms formulation consistency batch-to-batch.
- Procedure: Use a pycnometer.

IV. DISCUSSION

The development of herbal mouthwashes has gained significant attention due to increasing consumer preference for natural and safe oral care products. In this context, the incorporation of *Mimosa pudica*, a plant known for its antimicrobial, anti-inflammatory, and astringent properties, presents a promising approach for oral hygiene formulations. The high tannin content in certain herbal extracts can, however, reduce bioavailability and impart an astringent taste, potentially limiting their acceptability. Casein, a milk-derived protein, has been reported to bind and precipitate tannins, effectively reducing their adverse effects while preserving the therapeutic efficacy of bioactive compounds. This synergistic combination allows for enhanced palatability and user compliance, which are critical factors in daily oral care routines.

Recent studies have highlighted the role of *Mimosa pudica* in inhibiting common oral pathogens such as *Streptococcus mutans* and *Porphyromonas gingivalis*, suggesting its potential in preventing dental caries and periodontal diseases. Meanwhile, casein's tannin-binding capacity not only mitigates bitterness but may also improve the stability of phenolic compounds, allowing the mouthwash to retain its antimicrobial potency over extended storage periods. Additionally, the use of natural ingredients reduces the risk of side effects associated with synthetic chemicals, such as mucosal irritation or altered oral microbiota.

Despite these promising attributes, the formulation requires careful optimization to maintain the balance between tannin reduction and bioactive efficacy. Further in vitro and clinical studies are essential to evaluate long-term safety, efficacy, and sensory acceptability. Overall, the combination of *Mimosa pudica* and casein offers a novel and scientifically grounded strategy for developing an effective herbal mouthwash with improved taste and functional benefits.

V. CONCLUSION AND FUTURE SCOPE / RECOMMENDATIONS

Conclusion:

The development of a herbal mouthwash combining *Mimosa pudica* and casein represents a promising advancement in natural oral care solutions. *Mimosa pudica* contributes antimicrobial, anti-inflammatory, and astringent properties, targeting oral pathogens and supporting oral health, while casein effectively binds tannins, reducing bitterness and enhancing the palatability of the formulation. This combination addresses both therapeutic efficacy and user compliance, making it a viable alternative to conventional chemical-based mouthwashes. The natural origin of the ingredients also minimizes potential side effects, supporting long-term safety and acceptance among consumers.

Looking forward, further research is needed to optimize the formulation in terms of concentration, stability, and shelf-life. Detailed in vitro and in vivo studies, including clinical trials, are essential to validate its antimicrobial efficiency, tannin-binding capacity, and overall oral health benefits. Additionally, exploring the synergistic interactions between *Mimosa pudica* phytochemicals and casein could open avenues for enhancing bioavailability and functional activity. There is also potential for incorporating other complementary herbal extracts or natural agents to broaden the spectrum of antimicrobial action and improve sensory attributes. Ultimately, this formulation could pave the way for innovative, safe, and effective herbal mouthwashes, catering to the growing demand for natural oral hygiene products.

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