

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

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Abstract: The development of effective, biocompatible oral care formulations has driven growing interest in plant-derived compounds as alternatives to conventional chemical mouthwashes. *Mimosa pudica*, a medicinal plant known for its antimicrobial, antioxidant, and astringent properties, contains a high concentration of tannins that may influence both its therapeutic activity and formulation behavior. This review explores the development of a novel herbal mouthwash combining *Mimosa pudica* extract with gelatin as a functional excipient to reduce tannin content while preserving key bioactive components. Gelatin, a natural protein with known binding affinity for polyphenols, serves as a clarifying and complexing agent capable of modulating tannin levels through protein–tannin interactions. The review examines the extraction methods for *Mimosa pudica*, mechanisms of tannin–gelatin complex formation, and the implications of tannin reduction on the sensory, stability, and antimicrobial characteristics of the mouthwash. Particular emphasis is placed on optimizing the gelatin concentration to achieve an ideal balance between reducing undesirable tannin-related bitterness and astringency while maintaining the plant's therapeutic potential. Additionally, formulation parameters such as pH, preservative compatibility, and organoleptic properties are discussed in relation to ensuring product safety and consumer acceptability. Further, the antimicrobial efficacy of *Mimosa pudica*—targeting oral pathogens such as *Streptococcus mutans*—is evaluated before and after tannin reduction to assess the impact of gelatin treatment. Preliminary findings suggest that controlled tannin complexation can improve palatability without significantly diminishing biological activity. The review underscores the potential of integrating natural extracts with biopolymer-based modifiers in oral hygiene formulations and highlights future directions, including advanced characterization techniques and clinical validation. Collectively, this work supports the development of a safe, effective, and consumer-friendly herbal mouthwash utilizing *Mimosa pudica* and gelatin as a promising tannin-reduction strategy.

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

I. INTRODUCTION

The increasing preference for natural, biocompatible, and safe oral hygiene products has encouraged significant research into herbal formulations as alternatives to conventional chemical mouthwashes. Synthetic agents such as chlorhexidine, although effective, are associated with drawbacks including tooth staining, altered taste perception, and mucosal irritation, prompting consumers and researchers to explore gentler yet effective plant-based solutions. Herbal extracts are valued for their rich phytochemical profiles, offering antioxidant, anti-inflammatory, and antimicrobial activities essential for maintaining oral health. Within this context, *Mimosa pudica* has attracted notable attention due to its long-standing use in traditional medicine and its diverse array of bioactive compounds. The plant demonstrates potential effectiveness against oral pathogens, including those responsible for dental caries and periodontal diseases. Despite its therapeutic promise, challenges arise from its naturally high tannin concentration, which can lead to bitterness, astringency, and formulation instability when used in mouthwash preparations. Addressing these limitations is crucial for maximizing consumer acceptability and effectiveness. Therefore, understanding the unique chemical composition of *Mimosa pudica* and the impact of tannins on oral formulations forms an essential foundation for developing optimized herbal mouthwashes. 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.

Phytochemical 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.

Gelatin as a Natural Agent for Tannin Reduction in Herbal Formulations

Gelatin, a natural protein derived from collagen, offers a promising solution to the tannin-related limitations of *Mimosa pudica* extract when incorporated into mouthwash formulations. Its ability to interact with and bind tannins through protein–polyphenol complexation makes it effective in selectively reducing tannin content while retaining essential therapeutic compounds. This complexation not only lowers bitterness and astringency but also improves the clarity, stability, and overall sensory appeal of the formulation. Gelatin’s wide acceptance in pharmaceutical and food industries highlights its biocompatibility, safety, and functional versatility. By forming insoluble complexes with tannins, it facilitates partial removal without significantly compromising antimicrobial activity. This

approach enables a balanced formulation wherein the extract maintains its beneficial effects while becoming more suitable for routine oral use. Furthermore, understanding factors such as gelatin concentration, pH influence, and interaction kinetics is critical for optimizing tannin reduction. This review therefore explores the integration of gelatin with *Mimosa pudica* extract as a strategic method for developing an effective, consumer-friendly herbal mouthwash, highlighting key formulation considerations and future research directions. 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.

Rationale for Developing a *Mimosa pudica*-Gelatin 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. Therapeutic Potential of *Mimosa pudica* in Oral Healthcare

Extensive literature highlights *Mimosa pudica* as a medicinal plant rich in alkaloids, flavonoids, tannins, and phenolic acids, with proven antimicrobial, anti-inflammatory, and astringent activities. Studies indicate its significant inhibitory effect on oral pathogens, especially *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Porphyromonas gingivalis*. The high tannin content contributes to its antibacterial activity, but also affects taste, mouthfeel, and acceptability. Several experimental studies report that the crude extract exhibits potent free-radical scavenging activity, supporting its role in reducing oxidative stress in oral tissues. Previous herbal mouthwash formulations using *Mimosa pudica* demonstrate promising antimicrobial outcomes but face challenges related to bitterness, turbidity, and instability, indicating the need for improved formulation strategies.

2. Tannins in Herbal Extracts: Benefits and Limitations

Tannins, classified into hydrolyzable and condensed types, contribute significantly to the pharmacological properties of herbal extracts. They inhibit microbial adhesion, precipitate proteins of bacterial cell walls, and reduce inflammation. However, high tannin concentrations give rise to astringency, precipitation in aqueous solutions, and poor organoleptic properties. Literature shows that excessive tannins adversely affect patient compliance, especially for long-term mouthwash use. Several authors stress the necessity of partial tannin removal to balance therapeutic efficacy with sensory acceptability. Techniques including adsorption, enzymatic hydrolysis, and complexation with biopolymers have been explored, but gelatin has emerged as one of the most efficient and natural tannin-complexing agents.

3. Gelatin as a Tannin-Complexing Biopolymer

Research confirms that gelatin, due to its high content of proline-rich regions, can effectively bind tannins via hydrogen bonding, hydrophobic interactions, and protein–polyphenol complexation. This interaction leads to the formation of stable complexes that precipitate out, allowing controlled tannin reduction. In the food and beverage industry, gelatin has long been used as a clarifying agent, and recent pharmaceutical studies extend this application to herbal formulations. Investigations show that gelatin treatment reduces bitterness and turbidity while retaining essential bioactive compounds. It offers advantages such as biodegradability, non-toxicity, and compatibility with oral care systems. Literature supports its potential use in creating clearer, more palatable, and stable herbal mouthwash formulations.

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.
- Gelatin – 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. Gelatin-Based solution preparation:

A crucial part of this formulation methodology involves the use of gelatin as a natural tannin-complexing agent. Gelatin molecules contain proline-rich peptides that have strong affinity for tannins, allowing the formation of protein–polyphenol complexes. Within a formulation context, the gelatin is incorporated into the *Mimosa pudica* extract under controlled conditions that facilitate the interaction. The complexation process results in partial precipitation of tannins and leads to a reduction in bitterness, astringency, and turbidity. The mixture is typically subjected to an additional clarification step—such as simple standing, settling, or filtration—to remove the gelatin–tannin complexes. Literature emphasizes that optimizing gelatin concentration is crucial for balancing tannin removal with retention of antimicrobial activity.

Batch 1 (Concentrations):

<i>Mimosa pudica</i>	Gelatin
3 - 5 ml	4 ml
3 - 5 ml	6 ml
3 - 5 ml	8 ml
3 - 5 ml	10 ml
3 - 5 ml	2 ml

Batch 2 (Concentrations):

<i>Mimosa pudica</i>	Gelatin
3 ml	5 ml
2 ml	5 ml
5 ml	5 ml
4 ml	5 ml
2.5 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 Gelatin 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:

Orgenoleptic Evaluation

- 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:

The agar diffusion method—commonly referenced in antimicrobial research—is widely used to evaluate the inhibitory potential of mouthwash formulations against oral microorganisms. For herbal formulations like *Mimosa pudica* mouthwash, this method helps determine whether the tannin-reduced extract retains significant antimicrobial action.

The agar diffusion method can be explained conceptually as follows:

1. Preparation of Microbial Lawn:

The selected oral microorganisms are distributed uniformly on a solid agar medium to create a bacterial lawn suitable for observing growth inhibition.
2. Application of Mouthwash Sample:

The formulated *Mimosa pudica*-gelatin mouthwash is placed onto the agar surface either in wells or on pre-placed discs. The sample gradually diffuses outward into the agar.
3. Incubation:

The agar plates are maintained under suitable conditions that support optimal microbial growth, allowing interaction between the sample and the bacteria.
4. Observation of Zone of Inhibition:

After the incubation period, the plate is examined for clear circular zones surrounding the sample application site.

 - A larger zone indicates stronger antimicrobial activity.
 - A smaller or absent zone suggests limited or no activity.
5. Interpretation:

The inhibitory effect of the mouthwash formulation is compared with:

 - Untreated controls,
 - Extract without gelatin treatment, and
 - Standard antimicrobial agents (varies across studies).

The results help determine whether the gelatin-assisted tannin reduction has altered or preserved the antimicrobial potential of *Mimosa pudica*. Literature suggests that moderate tannin removal does not significantly compromise antimicrobial efficiency, making this approach suitable for improving taste while retaining therapeutic benefits.

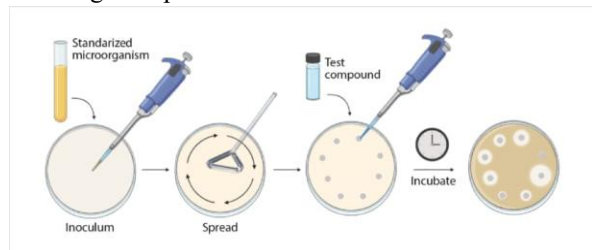


Fig. 1: Agar Diffusion Method

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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