Formulation and Evaluation of Herbal Mouthwash Using Mimosa Pudica and Egg Albumin as Tannin Reducing Agent

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***Abstract*—The present study was aimed at the formulation and evaluation of a herbal mouthwash using Mimosa pudica and egg albumin as a tannin-reducing agent. The formulation was prepared using herbal ingredients such as Neem, Tulsi, Aloe vera, peppermint oil, and clove oil for improved oral hygiene. Egg albumin was incorporated to reduce tannin-associated bitterness and improve the clarity and acceptability of mouthwash. The prepared formulations were evaluated for organoleptic properties, pH, stability, viscosity, and antimicrobial activity against oral pathogens. The results indicated that the herbal mouthwash showed satisfactory stability, acceptable physicochemical properties, and good antimicrobial activity. Thus, the formulated herbal mouthwash may serve as a safe, effective, alcohol-free, and natural alternative to conventional mouthwashes.**

***Index Terms*—Mimosa pudica, Herbal Mouthwash, Egg Albumin, Tannin-Reducing Agent, Antimicrobial Activity, Oral Hygiene, Polyherbal Formulation, Natural Oral Care, Stability Study, Herbal Antiseptic Mouthwash.**

I. INTRODUCTION

Oral hygiene plays an important role in maintaining overall health, and mouthwashes are commonly used to reduce microbial growth, plaque formation, and bad breath. Conventional mouthwashes containing chemicals such as chlorhexidine and alcohol may cause side effects like tooth staining, irritation, dryness, and altered taste on prolonged use. Therefore, herbal mouthwashes are gaining popularity as safer and natural alternatives.

Mimosa pudica is a medicinal plant known for its antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties, making it suitable for oral care formulations. However, the presence of tannins in the plant extract may produce bitterness and astringency. To overcome this limitation, egg albumin was used as a natural tannin-reducing agent due to its ability to bind with tannins and improve the taste and clarity of the formulation.

The present study focuses on the formulation and evaluation of a herbal mouthwash containing Mimosa pudica, Neem, Tulsi, Aloe vera, and other herbal ingredients. The formulated mouthwash was evaluated for physicochemical properties, antimicrobial activity, stability, and overall acceptability as a natural alternative to conventional mouthwashes.

II. BACKGROUND OF STUDY

Poor oral hygiene can lead to dental problems such as plaque formation, bad breath, gum infections, and tooth decay. Although chemical mouthwashes are commonly used for oral care, their prolonged use may cause side effects like tooth staining, irritation, dryness of mouth, and altered taste sensation. Therefore, there is an increasing demand for herbal mouthwashes that are safe, natural, and suitable for long-term use. Mimosa pudica is a medicinal plant known for its antimicrobial, anti-inflammatory, and antioxidant properties, making it useful for oral hygiene formulations. However, the presence of tannins in the plant extract may produce bitterness and astringency. Egg albumin has the ability to bind with tannins and reduce their undesirable effects, thereby improving the clarity and acceptability of the formulation.

III. AIM OF THE STUDY

To formulate and evaluate a herbal mouthwash using Mimosa pudica extract and egg albumin as a natural tannin-reducing agent for maintaining oral hygiene.

IV. OBJECTIVES

1. To formulate a herbal mouthwash using Mimosa pudica and other herbal ingredients.
2. To use egg albumin as a tannin-reducing agent to improve taste and clarity.
3. To evaluate the formulation for pH, viscosity, stability, and organoleptic properties.
4. To study the antimicrobial activity of the formulated mouthwash against oral pathogens.
5. To develop a safe, effective, alcohol-free, and natural alternative to conventional mouthwashes.

V. LITERATURE REVIEW

Herbal mouthwashes are widely used as natural alternatives to chemical mouthwashes because of their safety, effectiveness, and fewer side effects. Mimosa pudica is a medicinal plant known for its antimicrobial, anti-inflammatory, and antioxidant properties, making it useful in oral care formulations. Neem and Tulsi possess strong antibacterial activity against oral pathogens, while clove oil and peppermint oil provide antiseptic and refreshing effects. Aloe vera is also used for its soothing and healing properties.
Tannins present in herbal extracts may cause bitterness and astringency. Egg albumin acts as a natural tannin-reducing agent by interacting with tannins and improving the taste and clarity of the formulation. Previous studies have shown that herbal mouthwashes effectively control oral microorganisms and help maintain oral hygiene. Therefore, the present study was undertaken to formulate and evaluate a herbal mouthwash using Mimosa pudica and egg albumin.

VI. PLAN OF WORK

Phase I – Preformulation Studies
1. Literature review
2. Selection of herbal ingredients
3. Collection and authentication of plant materials

Phase II – Preparation of Extracts
4.Cleaning and shade drying of plant materials
5. Powdering of dried materials
6. Preparation of herbal extracts
7. Preparation of egg albumin solution and starch stabilizer

Phase III – Formulation Development
8. Preparation of herbal mouthwash batches (F1–F5)
9. Addition of flavoring and stabilizing agents
10. Filtration and storage of formulation

Phase IV – Evaluation Studies
11. Organoleptic evaluation
12. pH determination
13. Viscosity measurement
14. Tannin reduction study
15. Antimicrobial activity study
16. Stability study for 28 days

Phase V – Data Analysis and Documentation
17. Interpretation of results
18. Selection of optimized formulation
19. Conclusion and report preparation

MATERIALS USED IN HERBAL MOUTHWASH

1. Mimosa pudica
* Scientific Name: Mimosa pudica
* Family: Fabaceae
* Common Name: Touch-me-not plant / Sensitive plant
* Uses: Antimicrobial and anti-inflammatory agent
* Source: Leaves

2. Neem
* Scientific Name: Azadirachta indica
* Family: Meliaceae
* Common Name: Neem
* Uses: Antibacterial and antifungal agent
* Source: Neem leaves

3. Tulsi
* Scientific Name: Ocimum sanctum
* Family: Lamiaceae
* Common Name: Holy Basil
* Uses: Antimicrobial and antioxidant agent
* Source: Tulsi leaves

4. Aloe vera
* Scientific Name: Aloe barbadensis Miller
* Family: Asphodelaceae
* Common Name: Aloe vera
* Uses: Soothing and healing agent
* Source: Aloe leaf gel

5. Egg Abumin
* Scientific Name: Albumen ovi
* Family:
* Common Name: Egg white protein
* Uses: Tannin-reducing agent
* Source: Egg white

6. Peppermint Oil
* Scientific Name: Mentha piperita
* Family: Lamiaceae
* Common Name: Peppermint
* Uses: Flavoring and cooling agent
* Source: Peppermint leaves

7. Clove Oil
* Scientific Name: Syzygium aromaticum
* Family: Myrtaceae
* Common Name: Clove
* Uses: Analgesic and antiseptic agent
* Source: Clove buds

8. Lemon Extract
* Scientific Name: Citrus limon
* Family: Rutaceae
* Common Name: Lemon
* Uses: Flavoring and preservative agent
* Source: Lemon fruit

9. Glycerin
* Scientific Name: Glycerol
* Family: —
* Common Name: Glycerin
* Uses: Humectant and sweetening agent
* Source: Plant-derived oils

10. Honey / Stevia
* Scientific Name: Stevia rebaudiana
* Family: Asteraceae
* Common Name: Stevia
* Uses: Natural sweetener
* Source: Stevia leaves / Honey

11. Starch
* Scientific Name: Amylum
* Family:
* Common Name: Starch
* Uses: Stabilizing agent
* Source: Corn/Potato starch

12. Distilled Water
* Scientific Name: Aqua destillata
* Family:
* Common Name: Purified water
* Uses: Vehicle
* Source: Purified water source

CHEMICALS AND REAGENTS
Nutrient agar
Distilled water
Ethanol (if required for extraction)
Peptone
Ferric chloride

EQUIPMENTS USED
1. Beaker
2. Measuring Cylinder
3. Conical Flask
4. Glass Rod
5. Funnel
6. Whatman Filter Paper
7. Electronic Balance
8. pH Meter

9. Viscometer
10. Hot Plate / Magnetic Stirrer
11. Mortar and Pestle / Grinder
12. Incubator
13. Petri Plates
14. Micropipette / Dropper
15. Autoclave
16. Refrigerator

FORMULA BATCH:

Ingredients	F1	F2	F3	F4	F5
Mimosa pudica	3ml	3.5ml	2ml	2.5ml	4ml
Egg albumin + starch	2ml	2ml	1.5ml	3ml	3.5ml
Neem extract	2ml	2.5ml	1ml	3ml	3ml
Tulsi extract	2ml	1ml	2.5ml	1.5ml	3ml
Peppermint oil	0.1ml	0.09ml	0.1ml	0.12ml	0.08ml
Clove oil	0.05ml	0.045ml	0.049ml	0.051ml	0.047ml
Lemon juice	2ml	2ml	2ml	1.9ml	2ml
Aloe vera gel	2ml	2.5ml	2ml	2ml	3ml
Glycerin	5ml	4ml	3ml	4.5ml	5.5ml
Honey/stevia	2ml	2ml	2.5ml	2ml	3ml
Distilled water	Up to 100ml	Up to 100ml	Up to 100ml	Up to 100ml	Up to 100ml

VII. METHODOLOGY

1. Collection and Preparation of Plant Material

Fresh leaves of Mimosa pudica, Neem, and Tulsi were collected, washed thoroughly with distilled water, shade dried, and powdered using a grinder.

2. Preparation of Herbal Extract

The powdered plant materials were subjected to maceration using suitable solvent for 24–48 hours. The extracts were filtered using Whatman filter paper and concentrated.

3. Preparation of Egg Albumin Solution

Fresh egg white was separated carefully and diluted with distilled water to obtain egg albumin solution for tannin reduction.

4. Formulation of Herbal Mouthwash

The prepared herbal extracts were mixed with Aloe vera gel, glycerin, honey/stevia, starch, peppermint oil, clove oil, and lemon extract. Egg albumin was added slowly with continuous stirring. The final volume was adjusted using distilled water and filtered to obtain a clear mouthwash formulation.

5. Evaluation of Formulation

The formulated mouthwash was evaluated for:

* Organoleptic properties (color, odor, taste, clarity)
* pH determination
* Viscosity measurement
* Stability study
* Antimicrobial activity using agar well diffusion method

6. Stability Study

The prepared formulations were stored under suitable conditions and observed for 28 days for any changes in color, odor, pH, clarity, and precipitation.

PREPARATION PROCEDURE

*Preparation of Herbal Extracts:

Shade dry Mimosa pudica, Neem, and Tulsi leaves. Powder the dried material and macerate in 70% ethanol or warm water for 48 hours. Filter and concentrate.

*Preparation of EGG ALBUMIN Solution:

Dissolve EGG ALBUMIN (1% w/v) in distilled water. Add soluble starch in distilled water and heat gently until gel forms.

*Formulation:
Mix all aqueous extracts (Mimosa, Neem, Tulsi, Aloe vera) in a clean beaker.
Add vegetable glycerin, honey (or Stevia), and EGG ALBUMIN solution with gentle stirring.
Add peppermint oil, clove oil, and lemon extract while stirring continuously.
Make up the final volume to 100 mL with distilled water.
Mix thoroughly until a homogeneous solution is obtained

*Filtration and Storage:
Filter through muslin cloth or Whatman filter paper and store in amber-colored bottles under aseptic conditions.

FORMULATED HERBAL MOUTHWASH WAS EVALUATED USING THE FOLLOWING PARAMETERS

1. ORGANOLEPTIC EVALUATION-
- Color
- Odor
- Taste
- Clarity
- The formulation showed acceptable color, pleasant odor, and good appearance.

2. PH MEASUREMENT-
- The pH of the mouthwash was measured using a digital pH meter.
- Ideal pH for mouthwash: 5.5–6.5, safe for oral tissues.
- The formulation showed suitable pH for oral use.

3. VISCOSITY-
- Viscosity was determined using a Ostwald viscometer.
- Helps ensure mouthwash flows easily and feels comfortable during rinsing.

4. FOAMABILITY TEST-
- The mouthwash was shaken in a measuring cylinder, and foam formation was observed.

5. STABILITY TEST-
- The formulation was stored at room temperature for a specific period and observed for changes.

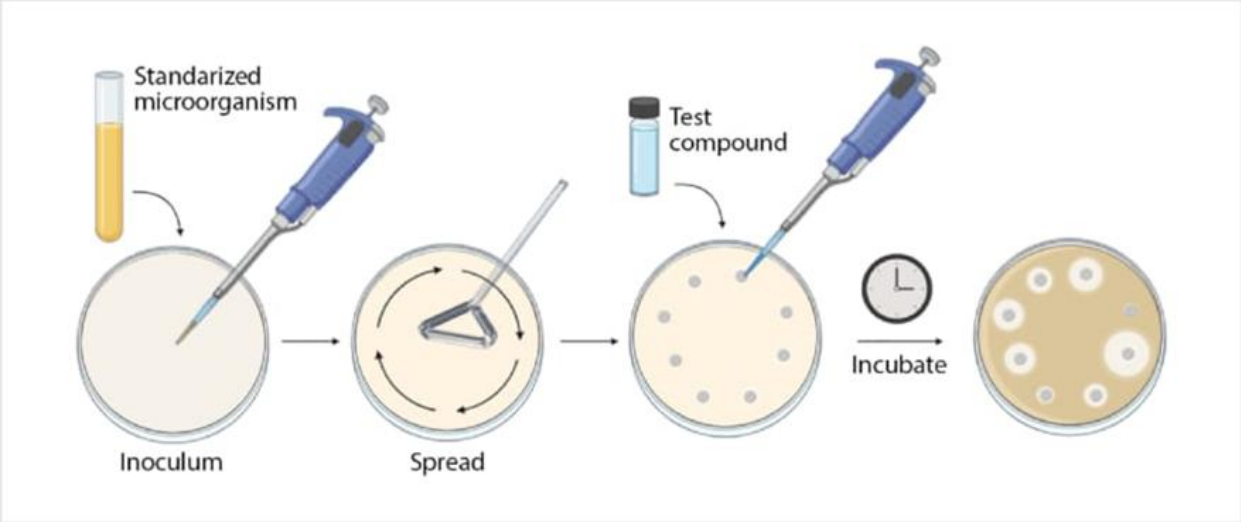
6. TANNIN REDUCTION TEST
Principle
The test was performed to determine reduction of tannin content using Moringa oleifera seed powder.
Observation
Reduction in tannin content was observed after treatment.

- Ferric Chloride Test

Principle
Ferric chloride test is used for the detection of phenols and tannins present in herbal extracts. Ferric chloride reacts with phenolic compounds and tannins to produce colored complexes such as blue, green, or dark coloration.
Procedure
1. Take small quantity of herbal extract in a test tube.
2. Add few drops of ferric chloride solution.
3. Observe the color change.
Observation
Formation of greenish-black or blue-black color indicates the presence of tannins and phenolic compounds in the extract.
Result
After the egg albumin treatment: The intensity of the dark coloration decreased significantly after treatment with egg albumin.

6. ANTIMICROBIAL ACTIVITY-
Performed using the agar well diffusion method against oral pathogens:
- *Streptococcus mutans*
- *Staphylococcus aureus*
- *Escherichia coli*
- *Candida albicans*

Results were observed by measuring the zone of inhibition after incubation.



Explanation of Agar Well Diffusion Method (as shown in figure):

1) Preparation of Inoculum:
A standardized microbial suspension (0.5 McFarland) of oral pathogens such as Streptococcus mutans, Staphylococcus aureus, Escherichia coli, and Candida albicans is prepared.

2) Inoculation of Agar Plate:
The bacterial culture is evenly spread over the surface of the solidified agar medium using a sterile swab to form a uniform lawn of microorganisms.

3) Well Formation:
Using a sterile cork borer or micropipette tip, small wells (6–8 mm) are made in the agar to hold the test solutions.

4) Addition of Test Compound:
The herbal mouthwash, along with controls, is carefully pipetted into the wells.

5) Incubation:
The plates are incubated at 37°C for 18–24 hours to allow microbial growth and diffusion of the test compound into the agar.

6) Observation of Results:
After incubation, clear circular zones appear around wells where microbial growth has been inhibited. These zones of inhibition indicate the antimicrobial potential of the mouthwash**.**

OBSERVED RESULT

The herbal mouthwash exhibited significant antimicrobial activity against oral microorganisms due to the presence of Mimosa pudica, Neem, Tulsi, and clove oil. Among all formulations, F1 showed better antimicrobial activity compared to other batches.

APPLICATIONS

1. Daily oral hygiene maintenance
2. Prevention of dental caries
3. Reduction of plaque and bad breath
4. Management of gingivitis and gum inflammation
5. Treatment of minor oral infections
6. Antimicrobial oral rinse against oral pathogens
7. Alcohol-free alternative for sensitive users
8. Suitable for long-term oral care use
9. Breath freshening and mouth cleansing
10. Herbal alternative to chemical mouthwashes
11. Educational and research applications
12. Eco-friendly and chemical-free oral care product

ADVANTAGES

100% herbal and chemical-free
Safe for long-term use
Good antimicrobial and anti-inflammatory activity
EGG ALBUMIN reduces tannin astringency and improves taste
Alcohol-free → no burning sensation
Easy and cost-effective to prepare
Eco-friendly and biodegradable

LIMITATIONS

1. Short shelf life due to natural ingredients

2. Variation in herbal composition and activity
3. Limited long-term stability studies
4. Taste and odor may vary among users
5. Possible microbial contamination during storage
6. Slower action compared to chemical mouthwashes
7. Egg albumin not suitable for vegan users
8. Large-scale production not evaluated

VIII. RESULT AND DISCUSSION

THE FINAL PRODUCT (F1)

1. Organoleptic Evaluation

Parameter	Observation
Color	Pale yellow
Odor	Sweet, pleasant odor
Taste	Refreshing taste
Clarity	Clear solution

Result:

The formulated herbal mouthwash showed acceptable organoleptic properties with pleasant appearance and good clarity.

2. Antimicrobial Activity

The antimicrobial activity of the formulated herbal mouthwash formulations (F1, F2, F3, F4, and F5) was evaluated by agar well diffusion method. The activity was determined by measuring the diameter of the zone of inhibition around each well after incubation.

Among all the formulations, formulation F1 showed the highest antimicrobial activity with a zone of inhibition of 20 mm, indicating strong inhibition against the test microorganism. Formulation F4 showed a zone of inhibition of 14 mm, while F3 and F2 showed 13 mm and 12 mm respectively. F5 showed the lowest antimicrobial activity with a zone of inhibition of 5 mm.

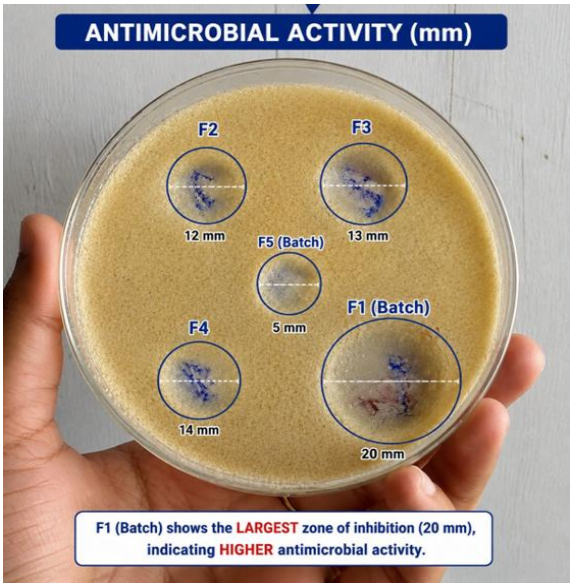
Order of Antimicrobial Activity:

F1 > F4 > F3 > F2 > F5

The larger zone of inhibition observed in F1 indicates that this formulation possesses better antimicrobial efficacy compared to the other formulations. The results suggest that the herbal ingredients present in formulation F1 exhibited significant antimicrobial potential and may be effective in controlling oral microorganisms.

Zone of Inhibition Table

Formulation	Zone of Inhibition (mm)
F1	20 mm
F2	12 mm
F3	13 mm
F4	14 mm
F5	5 mm



3. pH Measurement

The pH of the herbal mouthwash was found to be approximately 6.2, which is suitable for oral use.

Result:

The formulation was found safe and compatible with oral mucosa.

4. Viscosity

The formulation showed satisfactory flow property with moderate viscosity.

Result:

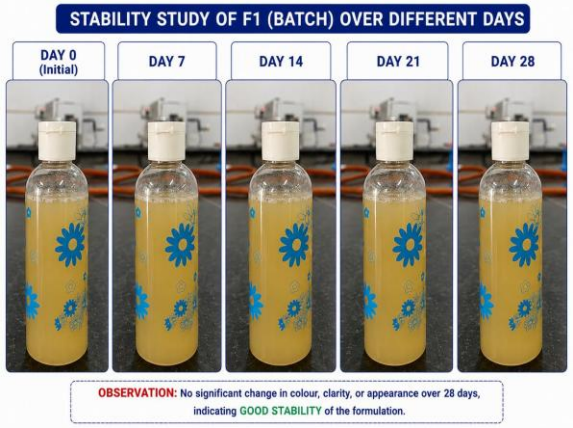
The mouthwash possessed suitable consistency for rinsing and oral application.

5. Stability Study

No significant changes were observed in color, clarity, odor, pH, or phase separation during the stability study period.

Result:

The formulation remained stable during storage at room temperature.



6. Physical Appearance

* Homogeneous liquid preparation
* Free from phase separation
* Elegant appearance
* Stable during storage

Result:

The formulation showed good physical stability and appearance.

7. Foamability Test

The formulation showed moderate and stable foam formation.

Result:

Good cleansing property and acceptable foamability were observed.

8. Ferric Chloride Test for Tannin Reduction

Observation:

Formation of light pale greenish-yellow coloration was observed in the test tube. Reduced intensity of color indicated decreased tannin content after treatment.

Result:

Ferric chloride test confirmed reduction of tannin content due to interaction of egg albumin with tannins, resulting in reduced astringency and improved palatability of mouthwash.

IX. CONCLUSION

The present study successfully formulated and evaluated a herbal mouthwash containing Mimosa pudica and egg albumin as a natural tannin-reducing agent. The formulation showed satisfactory physicochemical properties, acceptable stability, pleasant odor, refreshing taste, and good clarity. Egg albumin effectively reduced tannin-associated bitterness and improved the palatability of the formulation.

The herbal mouthwash exhibited significant antimicrobial activity against selected oral pathogens, indicating its potential in maintaining oral hygiene and preventing oral infections. Stability studies carried out for 28 days showed no significant changes in color, odor, pH, or appearance, confirming good stability of the formulation.

Overall, the formulated herbal mouthwash can be considered a safe, effective, economical, alcohol-free, and natural alternative to conventional chemical mouthwashes for routine oral care.

X. SUMMARY

The present study focused on the formulation and evaluation of a herbal mouthwash using Mimosa pudica and egg albumin as a tannin-reducing agent. The prepared formulations were evaluated for organoleptic properties, pH, viscosity, antimicrobial activity, stability, and tannin reduction. Among all formulations, F1 showed the highest antimicrobial activity with a zone of inhibition of 20 mm. The formulation showed pleasant odor, refreshing taste, suitable pH, good stability, and reduced tannin content. Overall, the herbal mouthwash was found to be effective, stable, alcohol-free, and suitable for maintaining oral hygiene.

XI. FUTURE SCOPE

1. Further optimization of herbal ingredient concentrations
2. Long-term stability studies for improved self-life
3. Clinical studies on larger populations
4. Comparative study with commercial mouthwashes
5. Development of alcohol-free commercial formulations
6. Use of alternative natural tannin-reducing agents

7. Incorporation of natural preservatives for extended stability
8. Development of eco-friendly packaging
9. Scale-up and commercialization of the formulation
10. Exploration of additional herbal extracts for enhanced activity
11. Evaluation of anti-plaque and anti-caries effectiveness
12. Development of vegan alternatives to egg albumin

MARKETED PRODUCTS

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