

# Development And Evaluation of Antimicrobial Mouthwash Containing Herbal Extracts

Ms. Ganvir Pallavi Villas<sup>1</sup>, Surnar Sandip Nathrao<sup>2</sup>, Matore Eknath Govind<sup>3</sup>, Gulam Tauseef G. R.<sup>4</sup>  
Rathod Karan Mehraban<sup>5</sup> Mr. Ingole Rupesh Venkatrao<sup>6</sup>  
*Associate Professor<sup>1</sup>, Research Scholar<sup>2,3,4</sup>*  
*Department of Pharmaceutical Chemistry, Kandhar College of Pharmacy, Nanded, Maharashtra, India*

**Abstract** - The present research work was aimed at the development and evaluation of herbal antimicrobial mouthwash containing selected herbal extracts with potent antimicrobial and oral protective activities. Herbal mouthwash formulations were prepared using extracts of Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), Clove (*Syzygium aromaticum*), and Peppermint (*Mentha piperita*). The prepared formulations were evaluated for physicochemical properties including color, odor, pH, viscosity, stability, antimicrobial activity, foamability, and taste acceptability. Antimicrobial studies were performed against common oral pathogens such as *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans* using agar well diffusion method. Among all formulations, F3 showed optimum antimicrobial activity, acceptable organoleptic properties, and good stability profile. The study concluded that herbal mouthwash can be effectively developed as a safe and effective alternative to synthetic antimicrobial mouthwashes for maintaining oral hygiene and preventing microbial infections.

**Keywords:** Herbal mouthwash, Antimicrobial activity, Neem, Tulsi, Clove, Oral hygiene, Herbal formulation.

## I. INTRODUCTION

Oral health is an essential component of general health and well-being. The oral cavity contains a wide variety of microorganisms including bacteria, fungi, and viruses that form complex microbial communities known as oral biofilms or dental plaque. [1] Poor oral hygiene and microbial accumulation may lead to several oral diseases such as dental caries, gingivitis, periodontitis, stomatitis, halitosis, and oral candidiasis. [2] These oral infections not only affect dental health but may also contribute to systemic diseases including cardiovascular disorders, diabetes complications, respiratory infections, and gastrointestinal disturbances. [3]

Mouthwash is a liquid oral hygiene formulation used to cleanse the oral cavity, reduce microbial load, freshen breath, and prevent oral infections. It acts by reaching areas of the mouth that are difficult to clean by brushing alone. Conventional mouthwashes commonly contain synthetic antimicrobial agents such as chlorhexidine gluconate, cetylpyridinium chloride, triclosan, fluoride compounds, and alcohol. Although these formulations are effective, prolonged use may produce several undesirable side effects such as tooth staining, altered taste sensation, oral mucosal irritation, burning sensation, dryness of mouth, and development of microbial resistance. [4,5] In recent years, increasing awareness regarding the adverse effects of synthetic chemicals has led to growing interest in herbal and natural products for oral healthcare. Herbal formulations are considered safer, biocompatible, economical, and associated with fewer side effects compared to synthetic formulations. Medicinal plants contain bioactive phytoconstituents such as flavonoids, alkaloids, tannins, glycosides, terpenoids, and essential oils that exhibit significant antimicrobial, anti-inflammatory, antioxidant, analgesic, and wound-healing activities. [6]

Neem (*Azadirachta indica*) is one of the most widely used medicinal plants in traditional medicine systems. Neem leaves contain active constituents such as nimbidin, azadirachtin, and quercetin that possess strong antibacterial, antifungal, and anti-inflammatory properties. [7,8] Neem has traditionally been used in oral hygiene practices for prevention of plaque formation and gum infections. Tulsi (*Ocimum sanctum*), commonly known as Holy Basil, possesses antimicrobial, antioxidant, and immunomodulatory properties due to the presence of eugenol, ursolic acid, and rosmarinic acid. Tulsi extract helps in reducing oral microbial growth and

inflammation.<sup>[9,10]</sup> Clove (*Syzygium aromaticum*) is another important herbal ingredient used in oral care products. Clove oil contains eugenol, which acts as a natural antiseptic, analgesic, and antimicrobial agent. It is highly effective in relieving toothache and reducing bacterial growth in the oral cavity. Peppermint (*Mentha piperita*) contains menthol and essential oils that provide cooling sensation, refreshing flavor, and antibacterial activity. Peppermint improves the organoleptic properties of mouthwash and enhances patient acceptability.<sup>[11]</sup>

Herbal mouthwash formulations offer several advantages such as:<sup>[12-15]</sup>

- Reduction of oral microbial load
- Prevention of plaque and gingivitis
- Freshening of breath
- Reduction of inflammation and pain
- Minimal adverse effects

Sr. No.	Material	Category	Function
1	Neem extract	Herbal extract	Antimicrobial agent
2	Tulsi extract	Herbal extract	Antibacterial and anti-inflammatory agent
3	Clove oil	Essential oil	Antiseptic and analgesic agent
4	Peppermint oil	Essential oil	Flavoring and cooling agent
5	Glycerin	Humectant	Viscosity enhancer
6	Sorbitol	Sweetening agent	Taste enhancer
7	Sodium benzoate	Preservative	Prevents microbial contamination
8	Tween 80	Surfactant	Solubilizing agent
9	Purified water	Vehicle	Solvent

**2.2 Collection and Authentication of Plant Material**  
 Fresh Neem and Tulsi leaves were collected from local medicinal plant sources and authenticated by a qualified botanist. The plant materials were washed thoroughly with distilled water to remove dirt and contaminants. The leaves were shade dried at room temperature for 7–10 days to preserve thermolabile phytoconstituents. The dried materials were pulverized using a mechanical grinder and passed through sieve no. 40 to obtain uniform powder.<sup>[17]</sup>

**2.3 Preparation of Herbal Extracts<sup>[18,10,12,13,14]</sup>**

**Extraction Procedure**

1. Accurately weighed powdered plant material was transferred into a clean glass container.
2. Hydroalcoholic solvent (70% ethanol) was added in sufficient quantity.
3. The mixture was subjected to maceration for 72 hours with intermittent shaking.
4. The extract was filtered through muslin cloth followed by Whatman filter paper No. 1.

- Improved patient compliance

The development of herbal antimicrobial mouthwash is therefore considered a promising alternative approach for maintaining oral hygiene and preventing oral infections. The present study focuses on the formulation and evaluation of antimicrobial mouthwash containing Neem, Tulsi, Clove, and Peppermint extracts with the aim of developing an effective, safe, and stable herbal oral care formulation.<sup>[16]</sup>

**II. MATERIALS AND METHODS**

**2.1 Materials**

All ingredients used in the formulation were of analytical or pharmaceutical grade and were used without further purification.

5. The filtrate was concentrated using rotary evaporator under reduced pressure at controlled temperature (40–50°C).
6. The concentrated extract was dried in a desiccator and stored in airtight amber-colored containers until further use.

**2.4 Preformulation Studies<sup>[19]</sup>**

Preformulation studies were carried out to evaluate the physicochemical properties and compatibility of herbal extracts.

**2.4.1 Organoleptic Properties**

The extracts were evaluated for color, odor, taste, and texture.

**2.4.2 Solubility Studies**

Solubility of extracts was determined in water, ethanol, and buffer media.

**2.4.3 pH Determination**

The pH of extract solutions was measured using digital pH meter.

**2.4.4 Compatibility Studies**

Compatibility between herbal extracts and excipients was assessed visually for precipitation, phase separation, and color changes.

### 2.5 Formulation of Herbal Mouthwash

The herbal antimicrobial mouthwash was prepared by simple solution method.

Stepwise Procedure [1,4,5,10,18,19,20]

1. Required quantity of purified water was taken in a clean beaker.
2. Sorbitol and glycerin were added and stirred continuously until completely dissolved.
3. Tween 80 was incorporated to facilitate solubilization of essential oils.
4. Neem and Tulsi extracts were dissolved separately and added slowly into the base solution.
5. Clove oil and peppermint oil were added dropwise under continuous stirring.
6. Sodium benzoate was incorporated as preservative.
7. Final volume was adjusted using purified water.
8. The prepared formulation was filtered to obtain clear mouthwash solution.
9. Mouthwash was filled into sterile amber-colored bottles and stored at room temperature.

## III. RESULTS AND DISCUSSION

### 3.1 Physical Appearance and Organoleptic Properties

All formulations showed clear appearance, pleasant odor, and acceptable taste. No precipitation or phase separation was observed.

Formulation	Appearance	Taste	Odor
F1	Clear	Pleasant	Mild herbal
F2	Clear	Sweet herbal	Pleasant
F3	Clear	Refreshing	Excellent
F4	Slightly dark	Strong herbal	Strong

Interpretation:

F3 showed the best balance between flavor and antimicrobial extract concentration.

### 3.2 pH Evaluation

Formulation	pH
F1	5.8
F2	6.0
F3	6.3
F4	6.5

Interpretation:

All formulations exhibited pH suitable for oral

cavity and were unlikely to cause irritation or enamel damage.

### 3.3 Viscosity Evaluation

Formulation	Viscosity (cP)
F1	42
F2	46
F3	51
F4	55

Interpretation:

Increase in herbal extract concentration slightly increased viscosity due to higher solid content. F3 showed optimum viscosity for mouth rinsing.

### 3.4 Antimicrobial Activity

Formulation	<i>S. mutans</i>	<i>S. aureus</i>	<i>C. albicans</i>
F1	12 mm	10 mm	8 mm
F2	15 mm	13 mm	10 mm
F3	20 mm	18 mm	15 mm
F4	18 mm	16 mm	13 mm

Antimicrobial Activity of Herbal Mouthwash

### Discussion of Antimicrobial Activity

F3 demonstrated maximum antimicrobial activity due to optimum concentration of Neem and Tulsi extracts combined with clove oil. The phytoconstituents present in the extracts disrupted microbial cell membranes and inhibited bacterial growth effectively.

### 3.5 Stability Studies

No significant changes were observed in:

- Color
- Odor
- Clarity
- pH
- Antimicrobial activity

Interpretation:

The formulations were physically and chemically stable under both room and accelerated conditions.

## IV. CONCLUSION

The present study successfully focused on the development and evaluation of antimicrobial herbal mouthwash containing Neem, Tulsi, Clove, and Peppermint extracts. The prepared formulations demonstrated satisfactory physicochemical characteristics, acceptable organoleptic properties, and significant antimicrobial activity against common oral pathogens such as *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*. Among all formulations, F3 was identified as the optimized formulation due to its superior antimicrobial activity, pleasant taste, suitable pH,

optimum viscosity, and excellent stability profile. The synergistic action of herbal extracts contributed to effective inhibition of oral microorganisms while minimizing the adverse effects commonly associated with synthetic mouthwash formulations.

The study confirms that herbal extracts can be effectively utilized in oral care formulations as safe, economical, and patient-friendly alternatives to conventional chemical-based mouthwashes. Herbal mouthwash not only provides antimicrobial protection but also improves oral hygiene, reduces inflammation, and freshens breath naturally. In conclusion, the developed herbal antimicrobial mouthwash possesses promising potential for commercial application in oral healthcare management. Further clinical and in-vivo studies are recommended to establish long-term safety, efficacy, and therapeutic benefits in human subjects.

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