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Formulation And Evaluation Of Herbal Mouthwash For Oral Hygiene

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ABSTRACT:

Oral hygiene plays a crucial role in maintaining overall health and the use of mouthwash is an important adjunct to regular brushing and flossing. However, concerns about the long-term effects of chemical-based mouthwashes, such as chlorhexidine, including staining, altered taste sensation and mucosal irritation have led to a growing interest in herbal alternatives. This research aims to formulate and evaluate a natural, alcohol-free herbal mouthwash using traditional medicinal plants with known oral health benefits. The selected herbs *Azadirachta indica* (neem), *Ocimum sanctum* (tulsi), *Salvadora persica* (miswak) and *Mentha piperita* (peppermint) were chosen based on their proven antimicrobial, anti-inflammatory and antioxidant properties.

KEYWORDS

Herbal mouthwash, oral hygiene, natural antimicrobials, phytotherapy, medicinal plants, *Azadirachta indica* (neem), *Ocimum sanctum* (tulsi), *Salvadora persica* (miswak).

INTRODUCTION:

Oral health is a fundamental aspect of general well-being with poor hygiene often linked to systemic conditions such as cardiovascular disease, diabetes and respiratory infections. Mouthwashes are widely used as an adjunct to mechanical cleaning for maintaining oral hygiene and preventing dental issues like plaque formation, gingivitis and bad breath. While synthetic mouthwashes, particularly those containing chlorhexidine and alcohol are effective, their prolonged use can lead to side effects such as tooth discoloration, mucosal irritation, altered taste sensation and microbial resistance. These drawbacks have encouraged a shift toward natural and plant-based alternatives that offer therapeutic benefits without harmful side effects. For centuries, medicinal plants have been employed in traditional systems of medicine for their antimicrobial, anti-inflammatory and wound-healing properties. Herbs such as *Azadirachta indica* (neem), *Ocimum sanctum* (tulsi) and *Salvadora persica* (miswak) have been extensively used in oral care practices across cultures.

Their bioactive compounds have demonstrated the ability to inhibit the growth of common oral pathogens and support gum health. Maintaining proper oral hygiene helps prevent common dental problems such as

dental caries, gingivitis, periodontitis and halitosis. Although mechanical cleaning methods like brushing and flossing are effective, they may not always be sufficient to reach all areas of the oral cavity. As a result, mouthwashes are often recommended as supportive agents to enhance oral cleanliness and reduce microbial load.



Fig: Mouthwash

In recent years, consumer preference has shifted toward safer, plant-based alternatives that offer therapeutic benefits without the undesirable effects of synthetic chemicals. Herbal formulations, rooted in traditional medicine are being revisited through modern scientific research for their potential to offer sustainable and side-effect-free solutions in oral care. Plants such as *Azadirachta indica* (neem), *Ocimum sanctum* (tulsi), *Salvadora persica* (miswak) and *Mentha piperita* (peppermint) have been widely recognized in Ayurveda and other traditional systems for their antimicrobial, anti-inflammatory, and astringent properties.

Maintaining oral hygiene requires a comprehensive approach, including regular brushing, flossing and the use of adjunctive products such as mouthwashes. Mouthwashes are widely used as they help in:

1. Freshening Breath (Halitosis)

- Herbs like mint, clove and fennel have natural deodorizing and antimicrobial properties.
- Helps neutralize odour-causing bacteria gently.

2. Fighting Gum Disease (Gingivitis, Periodontitis)

- Herbs like neem, tea tree oil and triphala are known to reduce inflammation, fight bacteria and promote gum healing.
- Regular use can help reduce gum bleeding and swelling.

3. Preventing Tooth Decay

- Ingredients like liquorice root and guava leaves have antibacterial properties that reduce plaque buildup.
- Some herbal mouthwashes also contain natural fluoride alternatives or mineral-rich extracts.

Limitations of Synthetic Mouthwashes:

- **Adverse Effects:** Mucosal irritation, tooth discoloration, and unpleasant taste.
- **Long-term Safety Concerns:** Risk of systemic toxicity with prolonged use.
- **Irritation:** Some synthetic mouthwashes contain alcohol, chlorhexidine or other strong chemicals that can irritate the mouth, tongue and gums.

Rise of Herbal Alternatives:

In response to these limitations, there has been a growing interest in herbal alternatives for oral care. Herbal mouthwashes utilize natural plant extracts with proven therapeutic properties, including:

1. Neem (*Azadirachta indica*):

- Potent antibacterial properties.
- Inhibits biofilm formation by disrupting bacterial cell walls.
- Acts as an anti-inflammatory and astringent agent.

2. Clove (*Syzygium aromaticum*):

- Contains eugenol, known for its antimicrobial, antifungal and analgesic properties.
- Reduces oral infections and provides relief from toothache.

3. Tulsi (*Ocimum sanctum*):

- Demonstrates significant anti-inflammatory activity.
- Inhibits the growth of oral pathogens like *Streptococcus mutans*.

Benefits of Herbal Mouthwashes:

- **Safety:** Free from harsh chemicals, reducing the risk of side effects.
- **Efficacy:** Combats oral pathogens effectively while maintaining oral tissue integrity.
- **Sustainability:** Environmentally friendly formulations using biodegradable ingredients.
- **Alignment with Traditional Medicine:** Leveraging the principles of Ayurveda, Unani and other traditional systems for holistic oral care.

Rationale Behind the Study:

The formulation and evaluation of a herbal mouthwash for oral hygiene is significant due to several reasons:

- **Consumer Demand for Natural Products:** As awareness of the potential risks of chemical ingredients grows, consumers are increasingly seeking natural alternatives. Developing a herbal mouthwash meets this demand and offers a safer, gentler option for oral care.
- **Herbal Efficacy:** The medicinal properties of various herbs have been well-documented in traditional medicine, with many herbs known for their antimicrobial, anti-inflammatory and healing effects on oral tissues.
- **Scientific Validation:** This study aims to provide scientific validation of the efficacy and safety of herbal mouthwash formulations, contributing to the growing body of literature on natural oral care solutions.

Key Challenges in Herbal Mouthwash Development:

While the potential benefits of herbal mouthwashes are clear, the formulation and commercialization of such products come with challenges. These challenges include:

• Herbal Ingredient Selection:

Not all herbs are suitable for inclusion in mouthwashes and their effectiveness can vary depending on factors such as extraction method, concentration and bioavailability. The selection of appropriate herbal ingredients must be based on scientific evidence of their antimicrobial and therapeutic properties. Moreover, the combination of herbs must be carefully considered to avoid interactions that may diminish their effectiveness.

• Formulation Stability:

One of the challenges in developing herbal mouthwashes is ensuring the stability of the herbal extracts over time. Many herbal compounds are prone to degradation when exposed to light, air or varying temperatures. Additionally, herbal extracts may require the addition of preservatives to prevent microbial contamination, while still maintaining the natural chemical-free appeal of the product.

History of Herbal Mouthwash:

The history of herbal mouthwash stretches back thousands of years, with documented use in multiple ancient civilizations where oral hygiene was maintained using natural plant extracts and rinses. As early as 2700 BCE, Chinese records reference the use of herbal infusions, including green tea and ginger, for treating mouth infections and bad breath. In Ayurvedic texts such as the Charaka Samhita (circa 1500 BCE), oral care routines included gandusha and kavala—oil or herbal decoction swishing techniques that used ingredients like neem, tulsi and triphala for their antimicrobial and anti-inflammatory effects. The Egyptians, around 1500 BCE, used rinses made from myrrh and natron mixed with water, believed to combat gingivitis and halitosis. The Greeks and Romans (circa 500 BCE – 200 CE) utilized vinegar and wine mixed with herbs such as mint and thyme as mouthwashes, emphasizing both medicinal and cosmetic purposes. The practice of using herbal formulations for oral hygiene has roots tracing back thousands of years. Around 2600 BCE, ancient Egyptian medical texts like the Ebers Papyrus documented the use of herbal mixtures, including myrrh and frankincense, for treating oral infections and gum diseases. In India, the Ayurvedic system, which emerged around 1500 BCE, emphasized the use of neem (*Azadirachta indica*), licorice root and clove oil for maintaining oral cleanliness and preventing dental ailments. Similarly, in ancient China, texts from the Zhou Dynasty (circa 1046–256 BCE) recommended herbal rinses made from honeysuckle and chrysanthemum for oral care. Scientific interest in herbal medicine reemerged strongly during the early 20th century, and by the late 1900s, numerous studies began evaluating the antimicrobial and anti-inflammatory properties of plant-based compounds for dental applications. In the 21st century, particularly after 2000, the formulation and clinical assessment of herbal mouthwashes gained momentum, driven by growing concerns over the side effects of synthetic oral care products. Today, herbal mouthwashes are increasingly studied for their potential to provide effective, natural alternatives in maintaining oral health.

MATERIAL AND EQUIPMENT'S:

➤ MATERIALS:

The following materials of grade or the best possible Laboratory Reagent (LR) were used as supplied by the manufacture.

Sr no.	Ingredients	Role
1	Neem	Antimicrobial
2	Tulsi	Antiseptic
3	Cinnamon Oil	Flavouring
4	Methyl Paraben	Preservative
5	Liquorice Powder	Sweetning Agent
6	Glycerol	Co-surfactant
7	Sodium Lauryl Sulphate	Surfactant
8	water	Vehicle

Table 1.1: List of Material Used

➤ **EQUIPMENT'S USED:**

Sr No.	Equipment	Purpose
1	Hot Plate with Magnetic Stirrer	Heating and mixing extracts
2	pH Meter	Measuring pH of mouthwash
3	Weighing Balance	Accurate weighing of ingredients
4	Beakers, Flasks, Measuring Cylinders	Mixing and measuring liquids
5	Filtration Unit (Whatman filter paper)	Filtration of extract formulation
6	Refrigerator	Storage of herbal extracts/formulation
7	Micropipette	Accurate measurement of small volumes
8	Glass Bottles / Vials	Storage and packaging of final mouthwash

Table 1.2: List of Equipment's Use**METHOD**➤ **Preparation of Ocimum Sanctum Extraction Process:**

1. Tulsi extract obtained by washing the leaves clean and sun dry them.
2. Grind the tulsi leaves into a fine powder using mortar and pestle. Ensure uniformly in particle size for efficient extraction.
3. Choose the appropriate solvent based on the desired compounds to be extracted. Acetone is commonly used for extracting bioactive compounds from tulsi.
4. The powder was macerated with 100% ethanol for 48 Hr in the beaker.
5. Then filtered that solution with the whatsmans filter paper.
6. The obtained extract used for formulation of herbal mouthwash.

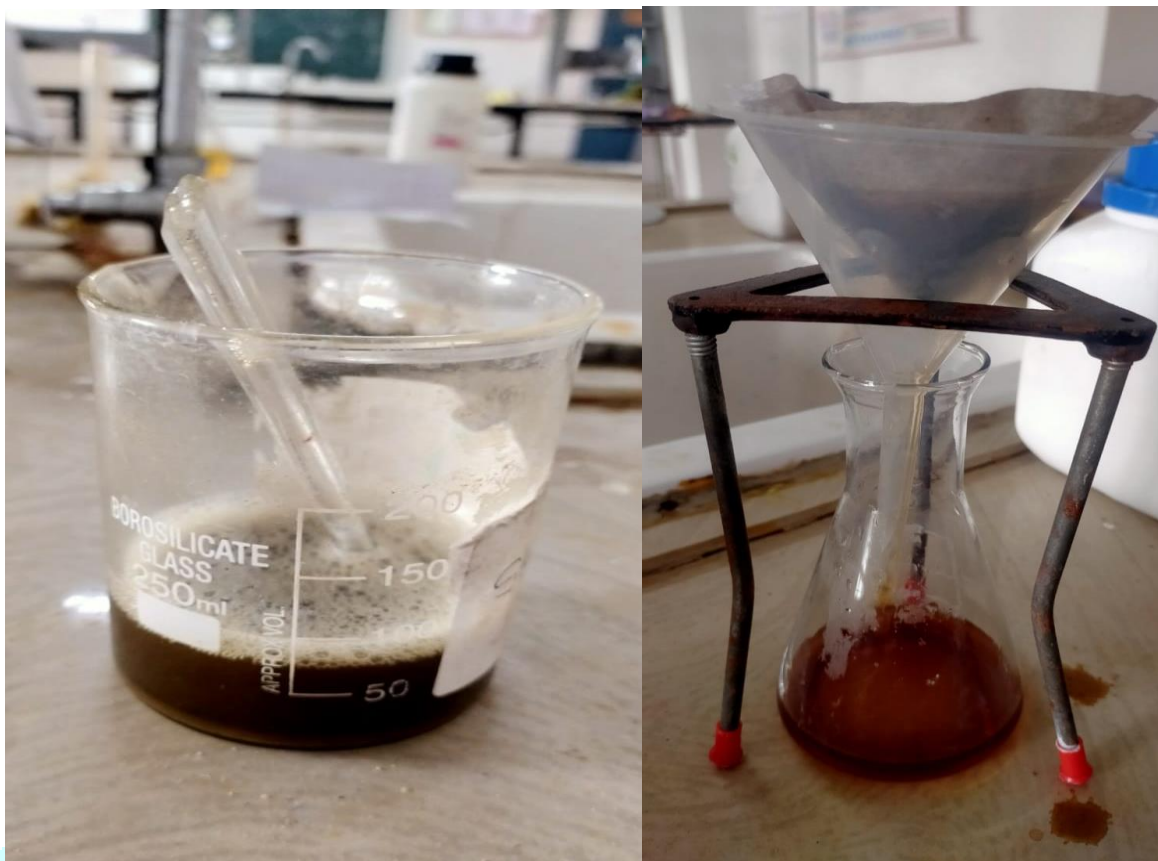


Fig: Ocimum Sanctum Extraction

➤ **Preparation of Herbal Mouthwash:**

- Preparing herbal mouthwash is a simple and rewarding process that requires just a few ingredients and some basic equipment.
- To start, select your preferred herbs, such as Neem, Tulsi extract, Glycerol, SLS, cinnamon oil, and liquorice root and dry them thoroughly to prevent mold and bacterial growth.
- Grind the dried herbs into a coarse powder using a mortar and pestle or coffee grinder, taking care to release the herbs' natural oils and flavour compounds.
- Then of that specific solution with the whatsmans filter paper to get clear solution of that preparation.
- Finally, store the mouthwash in a cool, dark place and use it by swishing it around your mouth for 30 seconds to one minute before spitting it out, taking care to avoid swallowing the liquid.
- **Storage Condition:** At room temperature in Airtight container.

FORMULATION TABLE:

Sr No.	Ingredients	F1	F2	F3	Functions
1	Tulsi Extracts	10 ml	10 ml	14.5 ml	Antibacterial
2	Liquorice powder	2 g	1.8 g	1.5 g	Sweetening Agent
3	Glycerol	6.5 ml	6.5 ml	6 ml	Co-surfactant
4	SLS	2.5 g	2.5 g	2 g	Surfactant
5	Distilled Water	Q.S	Q.S	Q.S	Vehicle
6	Cinnamon Oil	0.2 ml	0.1 ml	0.2 ml	Flavouring
7	Neem Powder	2.5 g	2 g	2.5 g	Antimicrobial
8	Methyl Paraben	0.3 g	0.1 g	0.3 g	Preservative

Table 1.3: Formulation Table

EVALUATION

❖ Organoleptic Evaluation:

In the evaluation of a herbal mouthwash, organoleptic properties such as colour and odour, play a crucial role in determining product acceptability and consumer compliance.

➤ Colour

The colour of the formulation is assessed visually under natural and artificial lighting conditions to ensure uniformity and stability over time. Any changes in colour may indicate chemical instability or contamination, which could compromise efficacy or safety.

➤ Odour

Odour is evaluated by direct smelling and compared with the natural aroma profile of the included herbal ingredients, such as clove, peppermint or neem. A pleasant and characteristic herbal scent is desirable, while any sour or rancid smell may point to degradation of volatile compounds.

➤ Taste

The taste is assessed through a controlled panel test, ensuring the formulation has a palatable flavour that is neither overly bitter nor excessively strong. The presence of natural sweeteners or mild flavouring agents is often used to enhance taste without compromising the therapeutic effects. These sensory evaluations are conducted repeatedly during stability studies to confirm that the formulation maintains its organoleptic integrity throughout its intended shelf life.

❖ pH Test:

pH of prepared herbal mouthwash was measured by using digital pH testing meter. The pH was calibrated using standard buffer solution about 1ml of mouthwash was weighed and dissolved in 50 mL of distilled water and its pH was measured.

The pH evaluation of a herbal mouthwash is an essential parameter in assessing its compatibility with the oral environment and ensuring product safety. A pH level within the range of 5.5 to 7.5 is generally considered ideal, as it helps maintain the natural pH balance of the oral cavity without causing enamel erosion or mucosal irritation. The pH of the formulation is measured using a calibrated digital pH meter. For accuracy, a small volume of the mouthwash typically around 50 mL is taken and the electrode of the pH meter is immersed in the solution at room temperature. The reading is recorded once the value stabilizes. This test is repeated at regular intervals during the product's stability study to monitor any shifts in acidity or alkalinity, which may indicate chemical degradation or microbial growth. Maintaining a stable pH is crucial, as significant deviations can impact the therapeutic effectiveness of herbal constituents and affect overall user comfort.

❖ Preservative Efficacy Test

The preservative efficacy test is conducted to evaluate the ability of the herbal mouthwash formulation to resist microbial contamination and maintain microbial stability throughout its shelf life. This test involves the intentional inoculation of the formulation with a known quantity of standard microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*.

Typically, a microbial suspension is added to the mouthwash sample under aseptic conditions, and the formulation is then stored at controlled temperature and humidity conditions. Samples are withdrawn at specific time intervals—commonly on days 7, 14, and 28—and are cultured to determine the reduction in viable microbial count.

A successful preservative system will demonstrate a significant decline or complete elimination of microbial growth over time, indicating that the formulation can effectively inhibit contamination. This test is especially important in herbal formulations, where natural ingredients may be more prone to microbial degradation without adequate preservation.

❖ Thermal Test:

Thermal stability testing is conducted to assess the resistance of the herbal mouthwash formulation to temperature variations, which can affect its physical, chemical, and microbiological stability.

In this test, the mouthwash is subjected to different temperature conditions—commonly including refrigeration (2-8°C), room temperature (25°C), and elevated temperature (40°C or 45°C)—over a defined period, usually ranging from one to seven days. Samples are withdrawn at regular intervals and evaluated for changes in physical appearance, such as colour, clarity, phase separation, odour and pH. A stable formulation should maintain its organoleptic properties and show no signs of degradation or precipitation under thermal stress. Thermal stability testing is particularly crucial for herbal products as natural ingredients can be sensitive to heat, potentially affecting their therapeutic properties.

RESULT AND DISCUSSION:

❖ Physical Evaluation:

The visually examination of the mouthwash is done by examine the physical characterization of the mouthwash and the results are given in the table below:

Sr NO.	Parameter	F1	F2	F3
1	Colour	Dark Brown	Dark Brown	Dark Brown
2	Odour	Fragrant	Fragrant	Fragrant
3	State	Liquid	Liquid	Liquid
4	Taste	Astringent sensation	Astringent sensation	Astringent sensation

Table 1.4: Physical Examination of Prepared Mouthwash

The mouthwash exhibited a dark brown colour due to the presence of clove and tulsi extract in the formulation. The colour of the mouthwash should remain consistent throughout the experimental phase to ensure the acceptability of the formulations. During the experimental phase the mouthwash formulations stored in the different temperature to examine the changes in formulations. First the formulation store in the refrigerator (12°C) retained a light brown colour, whereas those kept at room temperature (25°C) maintained a dark brown colour.

❖ pH Test:

Here I used the pH meter for measuring the pH value of formulation. We take 100 ml of mouthwash to determine the pH level of the mouthwash sample, 100 ml of the mouthwash was carefully measured and poured into a clean, dry beaker. A digital pH meter was used for accurate measurement. Before use, the pH meter was calibrated using standard buffer solutions (typically pH 4.0, 7.0, and 10.0) to ensure accuracy. Once calibrated, the electrode of the pH meter was gently rinsed with distilled water and then immersed into the mouthwash sample. The reading was allowed to stabilize for a few seconds before recording the value. The pH meter displayed a reading of pH 5.7, indicating that the mouthwash is slightly acidic. This level of acidity is commonly found in many mouthwash formulations, designed to reduce bacterial growth and promote oral hygiene. However, if the pH is too low, prolonged use may risk enamel erosion, emphasizing the importance of regular pH monitoring in oral care products.



Fig: pH Test

Sr no.	Formulation	pH
1	F1	5.2
2	F2	5.2
3	F3	5.7

Table 1.5: Result of pH Examination of Prepared Mouthwash

❖ Thermal Test:

Following exposure to various storage temperatures, the physical features of various mouthwash formulations. We evaluate the thermal stability of the herbal mouthwash formulation, a thermal test was conducted by storing two separate samples under different temperature conditions: one sample was kept in a refrigerator at 2–8°C, and the other at room temperature (approximately 25°C). Both samples were stored in clean, airtight containers to avoid contamination and evaporation. Over the course of one week, the samples were monitored for any physical or chemical changes, including alterations in colour and odour. At the end of the 7-day observation period, both samples were carefully examined and compared. The results indicated no significant changes in any of the observed parameters under either temperature condition. These findings suggest that the formulation is thermally stable at both refrigerated and room conditions over a short-term storage period. This stability is particularly important for consumer safety and shelf-life as it indicates the formulation can maintain its effectiveness and aesthetic properties under typical storage environments.

Sr No.	Room Temperature	Refrigerator temperature	Time period	Changes
1	25°C	2–8°C	1 week	No changes
2	25°C	2–8°C	1 week	No changes
3	25°C	2–8°C	1 week	No Changes

Table 1.6: Mouthwash Formulation Exposure to Different Temperature

DISCUSSION

The pH of an oral care product plays a critical role in maintaining oral health and preventing enamel erosion. In the present study, the formulated herbal mouthwash exhibited a pH of 5.7, which is well within the acceptable range for oral use. Maintaining a slightly acidic to neutral pH is essential to inhibit the growth of acidogenic bacteria and ensure compatibility with the oral mucosa. The stability of the pH over a 30-day period further suggests that the herbal ingredients used in the formulation do not undergo degradation or interaction that could alter the pH significantly over time. This stability indicates that the product is unlikely to cause irritation or disrupt the natural pH balance of the oral cavity. The thermal stability test was conducted to evaluate the product's ability to withstand different storage conditions without compromising its physical integrity or therapeutic properties. The herbal mouthwash retained its clarity, colour, and odour at all tested temperatures—4°C, 25°C, and 45°C—over a period of four weeks. These results suggest that the formulation is thermally stable and resistant to physical degradation when subjected to common environmental temperature fluctuations. The absence of phase separation or sedimentation under elevated temperature conditions further supports the robustness of the emulsifying and suspending agents used in the formulation.

SUMMARY

The present research focused on the formulation and evaluation of a herbal mouthwash using plant-based extracts known for their antimicrobial and anti-inflammatory properties. Key ingredients such as *Azadirachta indica* (neem), *Ocimum sanctum* (tulsi) and *Salvadora persica* (miswak) were selected due to their traditional use in maintaining oral health. These botanicals were extracted using standard techniques and incorporated into a mouthwash formulation, ensuring appropriate pH, viscosity and stability. The antimicrobial efficacy of the formulation was assessed against common oral pathogens including *Streptococcus mutans* and *Lactobacillus acidophilus*. Sensory characteristics, user acceptability and physicochemical parameters were also evaluated. The results demonstrated that the herbal mouthwash exhibited significant antibacterial activity comparable to that of standard chemical mouthwashes, without causing mucosal irritation or adverse taste.

CONCLUSION

The study successfully developed a herbal mouthwash with promising potential as a natural alternative to synthetic formulations for oral hygiene. The combination of medicinal plant extracts provided notable antimicrobial effects, supporting their role in the prevention of dental plaque and gingivitis. In addition to being effective, the herbal mouthwash was well-tolerated and flavoured by users due to its mild taste and absence of synthetic chemicals. These findings suggest that herbal formulations can be a safe, cost-effective and sustainable option for daily oral care. Future work should focus on clinical trials, long-term safety assessments and optimization of shelf life to further establish its commercial viability and therapeutic consistency. Additionally, thermal stability testing validates the formulation's resilience under various temperature conditions, which is essential for shelf-life prediction and quality control. Together, these evaluation parameters contribute to the development of an effective, stable and consumer-friendly herbal mouthwash. As the demand for safe and natural oral care products continues to grow, such formulations offer a viable alternative to conventional chemical-based mouthwashes, aligning traditional herbal knowledge with modern scientific validation. Continued innovation in this field may lead to the development of personalized herbal oral care products tailored to specific dental conditions. Overall, the successful formulation and evaluation of herbal mouthwash can play a significant role in promoting holistic oral health and reducing dependence on synthetic chemical agents.

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