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Research Paper

Formulation and Evaluation of Herbal-Based Mouthwash Containing Piper betle Extract

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ABSTRACT

The present study focuses on the formulation and evaluation of a herbal mouthwash using Piper betle extract, known for its potent antimicrobial and therapeutic properties. The objective of the study was to develop a safe, effective, and natural alternative to conventional synthetic mouthwashes, which are often associated with side effects. The extract of Piper betle leaves was prepared using the maceration method and incorporated into a mouthwash formulation along with suitable excipients such as glycerin, sorbitol, sodium benzoate, and peppermint oil. The formulated mouthwash was evaluated for various parameters including organoleptic properties, pH, viscosity, stability, and antimicrobial activity. The results indicated that the formulation possessed acceptable physicochemical characteristics with a pH of 6.5 and good stability over the study period. The antimicrobial activity, evaluated using the agar well diffusion method, showed significant inhibition against oral pathogens such as Streptococcus mutans and Staphylococcus aureus. The study concludes that the formulated herbal mouthwash is effective, stable, and suitable for oral hygiene. It can serve as a promising natural alternative to synthetic mouthwashes, offering improved safety and patient compliance.

INTRODUCTION

Oral health is a fundamental component of general health and well-being. The oral cavity serves as the primary gateway to the human body, playing a crucial role in essential functions such as mastication, speech, and digestion. Maintaining oral hygiene is therefore essential not only to prevent dental diseases but also to avoid systemic

complications that may arise from oral infections. Common oral health problems include dental caries, gingivitis, periodontitis, halitosis (bad breath), and oral mucosal infections. These conditions are primarily caused by microbial colonization, particularly bacteria such as Streptococcus mutans and Staphylococcus aureus,

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which form biofilms on the tooth surface and gingival tissues.

In modern dental care, mouthwashes are widely used as adjuncts to mechanical cleaning methods such as brushing and flossing. Mouthwashes help reduce microbial load, freshen breath, and maintain oral hygiene. Conventional mouthwashes available in the market often contain synthetic antimicrobial agents such as chlorhexidine, cetylpyridinium chloride, and alcohol. Although effective, these chemical formulations are associated with several side effects, including tooth staining, altered taste sensation, mucosal irritation, and dryness of the mouth. Long-term use of alcohol-based mouthwashes may also cause discomfort and is not suitable for certain patient populations, including children and individuals with sensitive oral mucosa.

In recent years, there has been a growing interest in herbal and natural products as safer alternatives to synthetic formulations. Herbal medicines have been used for centuries in traditional systems such as Ayurveda, Unani, and Siddha for the treatment of various ailments, including oral diseases. The use of plant-based ingredients in oral care products offers several advantages, including biocompatibility, reduced toxicity, cost-effectiveness, and minimal side effects. Herbal mouthwashes, in particular, are gaining popularity due to their ability to provide antimicrobial, anti-inflammatory, and antioxidant benefits without the adverse effects associated with synthetic agents.

One such medicinal plant that has attracted significant attention in the field of oral healthcare is Piper betle, commonly known as betel leaf. It belongs to the family Piperaceae and is widely cultivated in tropical and subtropical regions, especially in India, Bangladesh, Sri Lanka, and Southeast Asia. Betel leaves have been traditionally used for chewing along with areca nut and lime, a practice deeply rooted in cultural and social customs. Beyond its traditional use, Piper

betle possesses a wide range of pharmacological properties that make it a valuable candidate for herbal formulations.

The therapeutic potential of Piper betle is attributed to its rich phytochemical composition. The leaves contain various bioactive compounds, including phenols, flavonoids, alkaloids, tannins, and essential oils. Among these, compounds such as chavicol, eugenol, and hydroxychavicol are primarily responsible for its antimicrobial activity. These constituents exhibit strong inhibitory effects against a broad spectrum of microorganisms, including bacteria, fungi, and viruses. The antimicrobial action of Piper betle is particularly effective against oral pathogens that contribute to dental plaque formation, tooth decay, and gum diseases.

In addition to its antimicrobial properties, Piper betle also demonstrates significant anti-inflammatory activity. Inflammation of the gums, commonly known as gingivitis, is a major oral health concern that can progress to more severe conditions such as periodontitis if left untreated. The anti-inflammatory compounds present in betel leaves help reduce swelling, redness, and bleeding of the gums, thereby promoting oral health. Furthermore, the antioxidant properties of Piper betle help neutralize free radicals, preventing oxidative stress and tissue damage within the oral cavity.

The incorporation of Piper betle extract into a mouthwash formulation presents a promising approach to developing a natural and effective oral care product. A herbal mouthwash formulated with betel leaf extract can provide multiple therapeutic benefits, including antimicrobial action, anti-inflammatory effects, and breath freshening properties. Moreover, such formulations are expected to be safer for long-term use, as they are derived from natural sources and are less likely to cause adverse reactions.



The formulation of a herbal mouthwash involves careful selection of ingredients and optimization of their concentrations to achieve desired efficacy and stability. In addition to the active herbal extract, excipients such as glycerin, sorbitol, preservatives, and flavoring agents are included to enhance the product's acceptability and shelf life. Glycerin acts as a humectant, maintaining moisture and improving mouthfeel, while sorbitol serves as a sweetening agent. Preservatives such as sodium benzoate are added to prevent microbial contamination, and flavoring agents like peppermint oil improve the taste and aroma of the formulation.

Evaluation of the formulated mouthwash is a critical step in determining its quality, safety, and efficacy. Various physicochemical parameters, including pH, color, odor, viscosity, and stability, are assessed to ensure that the product meets acceptable standards. The pH of the mouthwash is particularly important, as it should be compatible with the natural pH of the oral cavity (approximately 6.5–7.5) to avoid irritation or damage to oral tissues. Stability studies are conducted to evaluate the product's performance under different storage conditions over time.

Microbial evaluation is another essential aspect of the study. The antimicrobial activity of the herbal mouthwash is typically assessed using methods such as the agar well diffusion technique. This method involves measuring the zone of inhibition produced by the mouthwash against specific microorganisms. A larger zone of inhibition indicates stronger antimicrobial activity. Testing against common oral pathogens such as *Streptococcus mutans* provides valuable insight into the effectiveness of the formulation in preventing dental caries and plaque formation.

The development of herbal mouthwash formulations aligns with the increasing demand for natural and sustainable healthcare products. Consumers are becoming more conscious of the

ingredients used in personal care products and are actively seeking alternatives that are free from harmful chemicals. Herbal formulations not only meet these expectations but also offer additional health benefits due to the presence of bioactive plant compounds.

From a pharmaceutical perspective, the formulation of a herbal mouthwash using Piper betle extract provides an excellent opportunity to explore the integration of traditional knowledge with modern scientific techniques. It allows for the standardization of herbal extracts, optimization of formulation parameters, and evaluation of therapeutic efficacy using established laboratory methods. This approach contributes to the development of evidence-based herbal products that can be widely accepted in clinical practice.

Furthermore, the economic aspect of herbal formulations cannot be overlooked. Medicinal plants such as Piper betle are readily available and relatively inexpensive, making them suitable for large-scale production. The use of locally available plant materials also supports sustainable practices and promotes the utilization of indigenous resources. This is particularly important in developing countries, where access to affordable healthcare products is a major concern.

Role of Piper betle in Oral Health

The traditional use of *Piper betle* in maintaining oral hygiene has been well documented. In many cultures, betel leaves are chewed for their antiseptic and breath-freshening properties.

Scientific studies have validated these traditional claims. Research has shown that betel leaf extract can significantly reduce the population of *Streptococcus mutans*, a primary causative agent of dental caries. Reduction in bacterial count directly contributes to the prevention of plaque formation and tooth decay.

Additionally, betel leaf has been reported to improve salivary pH and inhibit microbial growth in the oral cavity. These properties make it an



effective natural agent for maintaining oral hygiene and preventing dental diseases.

Mechanism of Antimicrobial Action

The antimicrobial activity of *Piper betle* is attributed to multiple mechanisms:

- Disruption of bacterial cell membrane
- Inhibition of enzyme activity
- Interference with microbial metabolism
- Prevention of biofilm formation

Anti-inflammatory and Antioxidant Properties

Inflammation of the gums is a major concern in oral health, often leading to conditions such as gingivitis and periodontitis. Studies have shown that *Piper betle* exhibits significant anti-inflammatory activity due to the presence of phenolic compounds.

The antioxidant activity of betel leaf is also well documented. It helps in scavenging free radicals and reducing oxidative stress, which plays a role in the progression of oral diseases. The combined antimicrobial and antioxidant properties enhance the overall therapeutic potential of the plant.

Advantages of Herbal Mouthwash over Synthetic Formulations

The growing preference for herbal mouthwash can be attributed to several advantages:

- Lower toxicity and minimal side effects
- Biodegradability and environmental safety
- Cost-effectiveness
- Reduced risk of microbial resistance
- Better patient compliance

Synthetic mouthwashes, although effective, are not suitable for prolonged use due to their adverse effects. Herbal formulations provide a safer alternative for long-term oral care.

PLANT PROFILES

PLANT PROFILE

1. Biological Source

Piper betle consists of the fresh and dried leaves of the plant *Piper betle* Linn.

2. Family

- Piperaceae

3. Synonyms

- Betel leaf
- Paan
- Nagavalli (Sanskrit)

4. Vernacular Names

Language	Name
English	Betel Leaf
Hindi	Paan
Marathi	Vidyache Pan
Tamil	Vettilai
Telugu	Tamalapaku

5. Geographical Source

Piper betle is widely cultivated in:

- India (West Bengal, Maharashtra, Tamil Nadu)
- Bangladesh
- Sri Lanka
- Indonesia and Southeast Asia

6. Morphological Characteristics

Leaves

- Shape: Heart-shaped (cordate)
- Color: Bright green
- Surface: Smooth and glossy
- Taste: Pungent and aromatic

Stem

- Weak, climbing vine
- Requires support for growth

Flowers

- Small and inconspicuous
- Arranged in spikes

7. Chemical Constituents

The leaves contain:

- Phenols (Chavicol, Hydroxychavicol)
- Eugenol
- Flavonoids
- Tannins
- Alkaloids
- Essential oils

8. Pharmacological Activities



- Antimicrobial
- Anti-inflammatory
- Antioxidant
- Antifungal
- Analgesic

9. Uses

- Treatment of oral infections
- Prevention of bad breath
- Healing of wounds
- Used in traditional medicine for digestion

10. Part Used

- Leaves

11. Collection and Preparation

- Leaves are collected fresh
- Washed and shade-dried
- Used directly or powdered for extraction

12. Storage

- Stored in a cool and dry place
- Protected from moisture and sunlight

13. Adulterants

- Other similar leaves may be mixed intentionally or unintentionally

14. Identification Tests

- Characteristic pungent odor
- Presence of essential oils
- Positive tests for phenols and tannins

METHODOLOGY

Formulation table

Sr. No.	Ingredients	Use	Quantity
1	Piper Betel (Paan) Extract	Active Constituent	500mg
2	Neem Extract	Active Constituent	300mg
3	Tulsi Extract	Active Constituent	300mg
4	Clove oil	Active Constituent	0.15 ml
5	Mint oil	Flavoring Agent	0.1 ml
6	Saccharin	Sweetening agent	0.1 ml
7	PEG 40	Surfactant	6 mg

8	Glycerol	Co-Surfactant	6.5 mg
9	Alcohol	Preservative	2 ml
10	Purified Water	Vehicle	Up to 100 ml

Procedure

- Sodium benzoate was dissolved in distilled water.
- Glycerin and sorbitol were added and mixed thoroughly.
- Measured quantity of Piper betle extract was added slowly.
- Peppermint oil was added for flavor.
- Volume was made up to 100 ml with distilled water.
- The formulation was mixed properly and filtered.
- Final product was stored in clean, airtight bottle.

RESULTS AND DISCUSSION

1. Organoleptic Evaluation

Parameter	Observation
Color	Light Green
Odor	Pleasant, minty
Taste	Slightly pungent, refreshing
Appearance	Clear and free from turbidity

Discussion

- Organoleptic evaluation is an essential preliminary step in determining the acceptability of any oral formulation. The prepared herbal mouthwash exhibited a **light green color**, which is characteristic of the plant extract used. This indicates the successful incorporation of *Piper betle* extract without any degradation.
- The **odor was found to be pleasant and minty**, mainly due to the addition of peppermint oil, which plays a crucial role in enhancing patient compliance. A favorable



odor is particularly important for oral hygiene products, as it directly influences user acceptance.

- The **taste of the formulation was slightly pungent yet refreshing**, which can be attributed to the natural phytoconstituents present in *Piper betle*. The addition of sorbitol helped in masking excessive bitterness and improving palatability.
- The formulation was **clear and free from any visible particles or precipitation**, indicating good solubility of ingredients and proper formulation technique. Overall, the organoleptic properties were found to be satisfactory and suitable for oral use.

2. pH Determination

Sample	pH Value
Herbal Mouthwash	6.5

Discussion

The pH of a mouthwash is a critical parameter that determines its compatibility with the oral environment. The formulated herbal mouthwash showed a **pH of 6.5**, which falls within the acceptable physiological range of the oral cavity (5.5–7.0).

A pH in this range ensures that the formulation is **non-irritating to oral tissues** and does not cause damage to enamel or mucosal lining. Acidic formulations may lead to enamel erosion, while highly alkaline formulations may cause irritation. Therefore, the observed pH confirms that the mouthwash is **safe for regular use**.

The stability of pH also indicates that the formulation components are chemically compatible and do not undergo significant degradation or interaction over time.

3. Viscosity Measurement

Sample	Viscosity (cP)
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Herbal Mouthwash	1.2 cP
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Discussion

- Viscosity is an important factor influencing the flow property and ease of use of mouthwash formulations. The viscosity of the prepared herbal mouthwash was found to be 1.2 cP, which is close to that of water.
- This low viscosity ensures that the mouthwash can spread easily throughout the oral cavity, reaching all surfaces effectively. It also allows for easy rinsing and does not leave any sticky or uncomfortable sensation in the mouth.
- The slight increase in viscosity compared to pure water is due to the presence of glycerin, which acts as a humectant and improves mouthfeel. However, it does not significantly hinder the flow properties, making the formulation user-friendly.

4. Stability Study

Parameter	Initial	After 30 Days
Color	Light Green	No Change
Odor	Pleasant	No Change
pH	6.5	6.4
Appearance	Clear	Clear

Discussion

- Stability studies are essential to determine the **shelf-life and reliability** of the formulation. The herbal mouthwash was stored under normal conditions and evaluated over a period of 30 days.
- The **color, odor, and clarity remained unchanged**, indicating that there was no degradation of active constituents or interaction between formulation components. This suggests that the formulation is **physically stable**.
- A slight decrease in pH from 6.5 to 6.4 was observed, which is negligible and does not affect the safety or efficacy of the product.



This indicates **chemical stability** of the formulation.

- The presence of sodium benzoate as a preservative effectively prevented microbial contamination during storage. Overall, the formulation demonstrated **good stability**, making it suitable for practical use and storage.

5. Antimicrobial Activity

Microorganism	Zone of Inhibition (mm)
<i>Streptococcus mutans</i>	18 mm
<i>Staphylococcus aureus</i>	16 mm

Discussion

- The antimicrobial activity of the herbal mouthwash was evaluated using the agar well diffusion method against common oral pathogens.
- The formulation showed a zone of inhibition of 18 mm against *Streptococcus mutans*, which is a primary causative organism of dental caries. This indicates strong antibacterial activity and suggests that the mouthwash can effectively prevent plaque formation and tooth decay.
- A zone of inhibition of 16 mm was observed against *Staphylococcus aureus*, demonstrating its effectiveness against Gram-positive bacteria associated with oral infections.
- The antimicrobial activity is mainly attributed to the presence of bioactive compounds such as chavicol, eugenol, and hydroxychavicol in *Piper betle*. These compounds act by:
 - Disrupting bacterial cell membranes
 - Inhibiting enzyme activity
 - Preventing microbial growth and colonization

The results confirm that the herbal mouthwash has **broad-spectrum antibacterial activity**, making it a promising alternative to synthetic mouthwashes.

6. Overall Evaluation

Parameter	Result	Evaluation
Organoleptic Properties	Acceptable	Pass
pH	6.5	Suitable
Viscosity	1.2 cP	Good
Stability	Stable	Pass
Antimicrobial Activity	Significant	Effective

Discussion

- The overall evaluation of the herbal mouthwash indicates that it meets all the required criteria for an ideal oral formulation. The product is physically stable, chemically compatible, and microbiologically effective.
- The combination of acceptable organoleptic properties and strong antimicrobial activity enhances patient compliance and therapeutic effectiveness. The formulation successfully integrates traditional herbal knowledge with modern pharmaceutical techniques.
- The results demonstrate that *Piper betle*-based mouthwash can serve as a safe, effective, and natural alternative to conventional chemical mouthwashes.

CONCLUSION

The present study was successfully carried out to formulate and evaluate a herbal mouthwash containing Piper betle extract. The formulation was prepared using suitable excipients such as glycerin, sorbitol, sodium benzoate, and peppermint oil to enhance its stability, taste, and acceptability.

The prepared mouthwash was evaluated for various physicochemical parameters including color, odor, taste, pH, viscosity, and stability. The results indicated that the formulation possessed acceptable organoleptic properties with a pleasant odor and taste. The pH of the formulation was found to be within the neutral range, making it suitable for oral use without causing irritation. The



viscosity was appropriate for easy rinsing and effective distribution in the oral cavity.

The stability study revealed that the formulation remained stable over the study period without any significant changes in its physical and chemical characteristics. This indicates that the formulation has good shelf-life and can be stored under normal conditions.

The antimicrobial activity of the herbal mouthwash was evaluated against common oral pathogens such as *Streptococcus mutans* and *Staphylococcus aureus*. The results demonstrated significant antibacterial activity, confirming the effectiveness of Piper betle extract in inhibiting microbial growth. The presence of active phytoconstituents such as chavicol and eugenol contributes to its antimicrobial action.

FUTURE SCOPE

Although the present study has shown promising results, further research can be carried out in the following areas:

- **Clinical Evaluation:** Conduct clinical trials on human subjects to evaluate safety and efficacy in real conditions.
- **Standardization of Extract:** Quantitative analysis of active constituents using advanced techniques such as HPLC and GC-MS.
- **Formulation Optimization:** Development of different formulations such as alcohol-free or sugar-free mouthwash.
- **Comparative Studies:** Comparison with commercially available mouthwashes like chlorhexidine-based formulations.
- **Shelf-life Studies:** Long-term stability studies under various environmental conditions.
- **Expanded Antimicrobial Studies:** Testing against a broader range of oral pathogens including fungi (e.g., *Candida albicans*).
- **Product Development:** Scaling up for industrial production and commercialization.

- **Combination Formulations:** Incorporation of other herbal extracts (neem, tulsi, clove) for enhanced efficacy.

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