



Preparation and Evaluation of Herbal Mouthwash Containing *Syzygium aromaticum* and *Ocimum sanctum* Extracts with Potential Antimicrobial Activity

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ARTICLE DETAILS	ABSTRACT
<p><i>Article history:</i> Received on 02 June 2024 Modified on 10 July 2024 Accepted on 15 July 2024</p> <p><i>Keywords:</i> Herbal mouthwash, <i>Syzygium aromaticum</i>, <i>Ocimum sanctum</i>, Antimicrobial activity.</p>	<p>The growing demand for natural, herbal alternatives in oral healthcare has led to the development of an antimicrobial mouthwash using clove (<i>Syzygium aromaticum</i>) and tulsi (<i>Ocimum sanctum</i>) extracts. This study aimed to prepare and evaluate a herbal mouthwash formulated with these extracts for its potential antimicrobial activity. Clove and tulsi are renowned for their bioactive compounds, including eugenol and flavonoids, known to exhibit potent antimicrobial effects. The mouthwash was prepared using standardized extracts of both herbs, ensuring the preservation of their active ingredients. Its antimicrobial efficacy was assessed against common oral pathogen such as <i>E. coli</i>, using well-established microbiological techniques like agar well diffusion. The results indicated significant antimicrobial activity with the herbal mouthwash. Furthermore, the formulation was evaluated for its physical properties, pH, viscosity and stability attributes. This study suggests that the herbal mouthwash containing clove and tulsi extracts can serve as a promising natural alternative for oral hygiene with effective antimicrobial properties, offering an eco-friendly option for consumers seeking alternatives to conventional oral care products.</p>

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INTRODUCTION

Herbal mouthwash has emerged as a compelling alternative to conventional oral care solutions, offering a blend of natural ingredients traditionally known for their medicinal properties. Unlike commercial mouthwashes that often rely on synthetic compounds and alcohol, herbal formulations harness the power of botanicals like neem, clove, tea tree oil, and chamomile. These ingredients are lauded not only for their antimicrobial and anti-inflammatory properties but also for their ability to promote overall oral health without the harsh side effects associated with chemical additives^[1]. The resurgence in interest towards natural remedies reflects a broader societal shift towards holistic health and sustainability. Herbal mouthwashes are increasingly favored by those seeking gentler yet effective means of maintaining oral hygiene. Scientific studies

support their efficacy in reducing plaque, combating bad breath, and soothing gum irritations. As consumers become more informed about the potential risks of long-term exposure to synthetic chemicals, herbal mouthwash stands out as a promising option that aligns with contemporary wellness trends while respecting traditional knowledge^[2,3].

Clove extract, derived from the dried flower buds of the *Syzygium aromaticum* tree, plays a pivotal role in the formulation of herbal mouthwash due to its multifaceted therapeutic properties. Its primary active compound, eugenol, is renowned for its potent antibacterial, antifungal, and analgesic effects. When incorporated into mouthwash, clove extract serves as a natural antimicrobial agent that helps combat oral pathogens responsible for conditions such as gingivitis and dental caries. Additionally,

eugenol's anti-inflammatory properties aid in reducing gum swelling and irritation, providing relief to individuals with sensitive gums. Moreover, clove extract contributes to the overall sensory experience of the mouthwash. Its distinctive aromatic profile imparts a refreshing and pleasant taste that enhances user compliance. The analgesic properties of eugenol also offer temporary relief from dental pain and discomfort, making it beneficial for individuals experiencing toothaches or oral sores. By integrating clove extract into herbal mouthwash formulations, manufacturers can leverage its holistic benefits to promote oral health naturally and effectively^[4].

Ocimum sanctum, commonly known as holy basil or tulsi, is renowned for its extensive therapeutic properties, making it a valuable addition to herbal mouthwash formulations. Its antibacterial, antiviral, and antifungal characteristics offer a broad-spectrum defence against oral pathogens, contributing significantly to oral hygiene. Research indicates that compounds such as eugenol and ursolic acid in *Ocimum sanctum* exhibit potent anti-inflammatory effects, which can help mitigate gum inflammation and reduce the risk of periodontal diseases^[5]. Moreover, the antioxidant properties of holy basil assist in neutralizing harmful free radicals in the mouth, promoting overall oral health. The herb's adaptogenic qualities also support stress reduction by balancing cortisol levels; this can indirectly benefit oral health since stress is a known factor exacerbating conditions like bruxism and canker sores. Incorporating *Ocimum sanctum* into an herbal mouthwash not only enhances its antimicrobial efficacy but also provides a holistic approach to maintaining oral health by leveraging the multifaceted benefits of this revered medicinal plant.

Honey has long been revered for its healing properties, extending far beyond its role as a natural sweetener. Its application in oral health, particularly within the formulation of herbal mouthwashes, presents a compelling case for its inclusion. Honey's inherent antibacterial and anti-inflammatory qualities make it an excellent candidate for promoting oral hygiene and treating various ailments such as gingivitis and mouth ulcers^[6]. Rich in enzymes like glucose oxidase, honey produces hydrogen peroxide when diluted with saliva, providing a potent

antimicrobial effect that can help combat harmful bacteria in the mouth. Additionally, honey's viscous nature aids in forming a protective barrier over sores or inflamed tissues, facilitating faster healing while reducing discomfort. Furthermore, honey contains antioxidants that can neutralize harmful free radicals in the oral cavity. Its soothing properties also help alleviate symptoms of dry mouth by stimulating salivary glands. Integrating honey into herbal mouthwash formulations not only enhances their therapeutic efficacy but also offers a natural alternative to chemical-laden commercial products^[7].

The aim of this study is to develop a natural, effective, and safe mouthwash formulation using herbal extracts of *Syzygium aromaticum* (clove) and *Ocimum sanctum* (holy basil) and to assess its antimicrobial properties. The goal is to evaluate the mouthwash's efficacy against common oral pathogens, such as bacteria, to promote oral health. The study also aims to investigate the physical properties, stability, and user acceptability of the herbal mouthwash.

MATERIALS AND METHODS

Materials

Extracts of *Syzygium aromaticum* and *Ocimum sanctum* were obtained from our laboratory. Honey was purchased from local market. All other chemicals used are of analytical grade.

Extraction of clove (*Syzygium aromaticum*)

Dried cloves are ground into a fine powder. Ethanol is added to the ground cloves, and the mixture is allowed to sit for several hours. The solvent is then filtered out, leaving behind the oil-solvent solution. The solvent is evaporated (under reduced pressure) to leave behind concentrated clove extract.

Extraction of tulsi leaves (*Ocimum sanctum*)

Fresh tulsi leaves were collected and dried under shadow. Then coarsely cut into small pieces and grind them into a fine powder. Ethanol was added to the ground leaves. The solvent dissolves the essential oils and other bioactive compounds from the leaves. After allowing the solvent to sit for a period of time (several hours), filter out the solid plant material. The solvent is then evaporated (vacuum evaporation), leaving behind a concentrated extract of tulsi.

Formulation of herbal mouthwash

Extracts of clove and tulsi were dissolving in 5ml of distilled water. Then add honey, sodium bicarbonate, methyl paraben, sodium chloride into above solution. Peppermint oil was used as flavouring agent. Then make up the volume up to 20ml by using distilled water (Table 1). This formulation was transferred into amber color close tight container.

Table 1: Formulation of herbal mouthwash

Ingredients	Quantity
Extract of <i>Syzygium aromaticum</i>	3ml
Extract of <i>Ocimum sanctum</i>	3ml
Honey	3ml
Methyl paraben	0.2gm
Sodium chloride	1gm
Sodium bicarbonate	0.5gm
Peppermint oil	1ml
Distilled water	Q.S.

Evaluation of herbal mouthwash

Physical evaluation

The physical evaluation (color, odor and taste) was carried out through visual and sensory inspection^[8-10].

pH

Prepared herbal mouthwash formulations were tested for pH using a digital pen-style pH meter. In order to assess pH, the pH meter's electrode was submerged in the mouthwash mixtures and left there until the level that was exhibited remained constant. Take note of the display's readings^[11-13].

Viscosity

The Brookfield viscometer was used to measure the viscosity using spindle number S64.25 mL of mouthwash was added to 50 mL of the clean glass beaker in order to determine the viscosity. In order to avoid touching the beaker's base, the spindle was dropped in the middle perpendicularly. Sample size, temperature, and pressure-variables that alter viscosity-were preserved. At room temperature and with the spindle running at 2.5 rpm, the viscosity was measured^[14-16].

Stability Studies

Stability studies were conducted to ensure that mouthwash formulations could be utilised and would maintain their identical qualities over

time. Stability tests on mouthwash formulation were carried out before antibacterial testing. The mouthwash's physical separation, homogeneity, and visual appeal were all assessed during the test. Mouthwash formulation was stored at various temperatures, such as 12°C and 25°C. After evaluating the appearance at different temperatures, the results were noted^[17,18].

Antimicrobial Study

The agar well diffusion experiment against *E. coli* was used to assess the test samples' capacity to inhibit pathogens. The spread plate method was used to inoculate the test microorganism onto Muller Hinton Agar (MHA) plates after they had been prepared and sterilised. Amikacin (used as a positive control) and mouthwash formulation were assessed. The agar was punched with wells that were 10 mm in diameter using a sterile maize borer. After the plates were incubated for 24 hours at 37°C, the antibacterial efficacy was evaluated by measuring the zone of inhibition (ZOI) with a Vernier calliper^[19-27].

RESULTS AND DISCUSSION

Physical Evaluation

The findings of physical tests of mouthwash formulation are displayed in Table 2.

Table 2: Physical evaluation

Parameter	Observation
Color	Greenish
Odor	Cool mint
Taste	Sweet

pH

The pH of mouthwash formulation was found to be 6.5. In the context of the herbal mouthwash formulation, the pH value of 6.5 indicates that the mouthwash is slightly acidic, which is within the acceptable range for oral care products. The pH of mouthwashes typically ranges from 4.5 to 7.5, with a neutral or slightly acidic pH being preferred to ensure it does not cause irritation to the mucous membranes of the mouth. A pH of 6.5 suggests that the mouthwash is gentle on the oral tissues, helping to maintain the natural balance of the oral cavity, preventing issues like dryness or irritation. Moreover, a slightly acidic pH can also contribute to the antimicrobial effectiveness of the formulation. Many oral pathogens, especially those responsible for plaque formation and gum disease, thrive in alkaline environments.

Viscosity

The dial reading revealed that the viscosity of the mouthwash was found to be 8.3 cP. The viscosity observed in the mouthwash formulation is a noteworthy result, indicating that the product has a moderate consistency. A product with this level of viscosity will flow easily from the bottle and spread across the mouth, ensuring effective coverage of the oral cavity.

Stability Studies

Physical stability research findings were displayed in Table 3. Change in color, odor, physical separation and the consistency of the mouthwash formulation was measured between 12°C and 25°C. It was observed that nothing has been changed in physical parameters after subjecting the mouthwash to two different temperatures.

Table 3: Stability Studies

Parameters	Temperature 12°C	Temperature 25°C
Color	Nothing has changed	Nothing has changed
Odor	Nothing has changed	Nothing has changed
Physical Separation	Nothing has changed	Nothing has changed

Antimicrobial Study

In contrast to amikacin, the study examines the antibacterial effectiveness of mouthwash formulation. Using techniques like the disc diffusion test, the antibacterial activity is evaluated against specific bacterial strains. Table 4 displays the results of the antimicrobial performance research. The zones of inhibition values were determined to be 25mm and 19mm for amikacin and mouthwash respectively.

Table 4: Zone of Inhibition

Sample	Zone of Inhibition (mm)
Amikacin (Standard) (30µg)	25
Herbal mouthwash (100mg)	19

The combined antimicrobial effect of *Syzygium aromaticum* (clove) and *Ocimum sanctum* (tulsi) in a mouthwash formulation holds significant potential for oral health. The antimicrobial actions of clove and tulsi may offer a broader spectrum of activity. The synergy between the two herbs could lead to enhanced antibacterial effects against common oral pathogens.

CONCLUSION

The exploration into herbal mouthwashes reveals promising avenues for promoting oral health through natural solutions. Herbal formulations, enriched with plant-based extracts, offer a holistic approach to dental hygiene by harnessing the antimicrobial, anti-inflammatory, and antioxidant properties of various herbs. This study highlights the efficacy of ingredients such as clove, tulsi and peppermint in reducing oral pathogens and maintaining fresh breath without the adverse effects often associated with chemical counterparts.

ACKNOWLEDGEMENT

The authors express their gratitude to the Pharmacognosy and Pharmaceutics Departments at Shree Santkrupa College of Pharmacy, Ghogaon for providing the resources required to conduct this study.

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