Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2022.943

Formulation, development, and evaluation of anti-inflammatory and antimicrobial effects of a novel polyherbal mouthwash—An *in vitro* study

Bhavana Garapati[1*], Jaiganesh Ramamurthy[1], Rajeshkumar Shanmugam[2]

[1]Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai

[2]Nanobiomedicine Lab, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai

***Corresponding author:** Bhavana Garapati, Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, 162 Poonamallee High Road, Velappanchavadi, Chennai, 600077. Tamil Nadu. Email: bhavanagarapati97@gmail.com

Submitted: 17 June 2022; Accepted: 24 July 2022; Published: 15 August 2022

ABSTRACT

Aim: The aim of this study was to prepare a polyherbal mouthwash and evaluate its antimicrobial and anti-inflammatory efficacy against commercially available herbal mouthwash. The objective was to signify whether the novel herbal combination (extracts of *Zingiber officinale* [ginger], *Curcuma longa* [turmeric], and *Syzygium aromaticum* [clove] 5% v/w) could be a better alternative to commercially available herbal mouthwashes.

Materials and Methods: An in vitro study was undertaken in which extracts of *Z. officinale* (ginger), *C. longa* (turmeric), *and S. aromaticum* (clove) 5% v/w were used. Seven different concentrations were prepared and tested against *Streptococcus mutans, Enterococcus faecalis, Candida albicans,* and *Staphylococcus aureus* in Mueller–Hinton agar medium. Plates were incubated aerobically at 37° C for 48 h, and the zone of inhibition was measured using a vernier caliper. Commercially available herbal mouthwash (Hiora) was used as a control group. The data were analyzed by descriptive analytics.

Results: Results showed that the efficacy of novel polyherbal mouthwash had comparatively less significant antimicrobial properties against the microorganisms as compared to the commercially available herbal mouthwash. The minimum inhibitory concentration was also found to be very high, that is, 100 µg/mL.

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Conclusion: There was no significant antimicrobial and anti-inflammatory effects for the polyherbal mouthwash as compared to commercially available herbal mouthwash (Hiora). Because this combination is readily available, it can be a cost-effective alternative to commercially available herbal mouthwashes.

Keywords: *clove; Curcuma longa, ginger, herbal mouthwash, Syzygium aromaticum, turmeric, Zingiber officinale.*

INTRODUCTION

Dental plaque can be defined as a specific but highly variable structural entity, resulting from sequential colonization of microorganisms on tooth surfaces, restorations, and other parts of oral cavity, composed of salivary components such as mucin, desquamated epithelial cells, debris, and microorganisms, all embedded in an extracellular gelatinous matrix Extensive research has shown that dental plaque is a major factor in the onset and progression of gingival and periodontal diseases.[1] Plaque control is critical for preventing gingivitis, dental caries, and halitosis-causing microorganisms. The commonly used tools or methods in the treatment of supragingival plaque are tooth brushing either mechanical or electrical, dental floss, or interdental brushing.[2,3] Other means of plaque control are chemical therapeutic agents such as mouthwashes, sprays, chewing gums, and varnishes, which aid in an effective home care.[2] Nevertheless, mouthwashes have been accepted as the simplest and easiest mode of oral hygiene aid.[2–4,5] This could be the main mode of oral cleansing in medically compromised patients and elderly where adequate oral hygiene maintenance could be a major concern.[6] Among chemical means, chlorhexidine (CHX) has been the most widely used mouthwash and is considered as the gold standard in dental practice for about three decades, but not without certain disadvantages such as taste perturbation, tooth discoloration, oral mucosal erosion, and unilateral or bilateral parotid swelling.[7] Keeping in mind the drawbacks of CHX mouthwashes, alternative antiplaque agents have been developed in recent years with the use of herbal products. Even though there are many commercially available herbal mouthwashes, there is still a need for natural products to minimize the side effects caused by chemical products.[8,9] "Ayurveda" (Ayur - Life and Veda - Science), the system of Indian medicine, has been used successfully for treating various systemic ailments.[1] Herbs have been the primary source of medication since the times of Charaka and Sushruta, and they have gained the trust of the Asian people. Naturally available herbs, such as tulsi, triphala, neem, honey, ajwain, and turmeric, have been commonly used either alone or in combination as safe and effective antibacterial agents.[10] Turmeric, scientifically known as *Curcuma longa,* and more commonly known as "haldi," possesses anti-inflammatory, antioxidant, and antimicrobial properties, as well as hepatoprotective, immune stimulant, antiseptic, antimutagenic, and many other properties.[11,12]

Ginger (*Zingiber officinale*) is used in traditional medicine for the treatment of many diseases such as inflammation, morning sickness in pregnancy, and many infectious diseases.[13-14.] It is shown that ginger extracts have profound antioxidant, antibacterial, anti-inflammatory, and antifungal effects.[15] Their effects on *Candida albicans* in vitro and utility in dental diseases have previously been reported;[16,17] however, little is known about its role as a mouthwash. Previous researches have shown that ginger extract has antimicrobial properties against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.[18,19]

Cloves (*Syzygium aromaticum*) are dried, aromatic, unopened floral buds from the Myrtaceae family. Clove is a natural antibiotic with broad antibacterial, antifungal, and antiviral activities.[20]

Dentists use it in bandages for minor wounds and as an analgesic in the treatment of painful and infective oral disorders. It is also used in medical and dental professions as an analgesic, antispasmodic, and general antiseptic.[21]

Plants have been used for medicinal purposes since the dawn of human civilization. Medicinal plants have been used for curing diseases in different traditional systems of medicine such as Ayurveda, Siddha, European, Tibetan, and Unani.[22] Due to its high cultural acceptability, compatibility with the human body, and less side effects, herbal therapy is still the mainstay of therapy in roughly 75–80% of people in many underdeveloped nations for their primary health care.[23] Thus, a potential supporting antimicrobial alternative with minimized side effects would be greatly valued to work on oral infections.[24,25] There is a need for an alternative medicine enmeshed within precious traditional Indian herbal therapy, which is efficient, safe, and economical. Hence, this study aims to find the antimicrobial and anti-inflammatory effects of a polyherbal (extracts of *Z. officinale* [ginger], *C. longa* [turmeric], and *S. aromaticum* [clove]) mouthwash when compared with a commercially available herbal mouthwash (Hiora).

MATERIALS AND METHODS

Preparation of mouthwash

Ingredients used

- 600 g turmeric powder
- 600 g ginger powder
- 600 g clove powder
- 50 µL peppermint oil
- 0.3 g sucrose
- 0.001 sodium benzoate
- 0.01 g sodium lauryl sulphate

Steps in preparation

The mouthwash was prepared using 600 g of turmeric powder, 600 g of ginger powder, 600 g of clove powder, distilled water, 0.3 g of sucrose, 0.001 sodium benzoate, clove oil, and 0.01 g of sodium lauryl sulphate. Silica nanoparticles were the main constituent, ethanol acted as a solvent to solubilize the ingredients, sodium benzoate as a preservative, and 50 µL of peppermint oil acted as a flavoring agent (Figure 1).

Antimicrobial activity

Antimicrobial action of the nanoparticles was checked against strains of *S. aureus*, *Streptococcus mutans*, *Enterococcus faecalis,* and *C. albicans*. The inhibition area was determined by Mueller–Hinton agar (MHA). MHA was prepared and sterilized at 120 lbs for 45 min. The media was poured onto the sterilized plates and left to solidify. The well cutter was used to cut the wells, and the test organisms

FIGURE 1. Novel polyherbal mouthwash preparation.

were swabbed. The plates were filled with mouthwash of various concentrations and incubated for 24 h at 37° C. The zone of inhibition was assessed after the incubation period.

Anti-inflammatory activity

Albumin denaturation assay

The anti-inflammatory function of *Solanum torvum* gel was assessed using the Mizushima and Kobayashi convention, with some modifications (Pratik Das [A4] et al., 2019)[26]. 0.45 mL bovine serum albumin (1 % aqueous solution) was applied to 0.05 mL *S. torvum* gel with various fixation (10 L, 20, 30, 40, and 50 L), and the pH of the mixture was acclimated to 6.3 using a small amount of 1 N hydrochloric acid. These samples were incubated at room temperature for 20 min before being heated in a water bath for 30 min at 55° C. After cooling the samples, the absorbance at 660 nm was measured spectrophotometrically. Diclofenac sodium was used as a control. Dimethyl sulfoxide was used as a monitor (Figure 2).

Percentage of protein denaturation was determined using the following equation:

% inhibition = (Absorbance of control − Absorbance of sample × 100) / Absorbance of control

RESULTS

Antimicrobial activity

The results indicated that the antimicrobial property of the mouthwash was effective in inhibiting the growth of *S. aureus, S. mutans, E. faecalis,* and *C. albicans* (Figures 3–6). At concentrations 25, 50, and 100 µL, *E. faecalis* had the highest zone of inhibition compared to the other three microorganisms. At 50 µL, *S. mutans* (11 mm) had a greater zone of inhibition than *S. aureus* (9 mm) and *C. albicans* (9 mm) (Table 1). At 25 µL, all the three

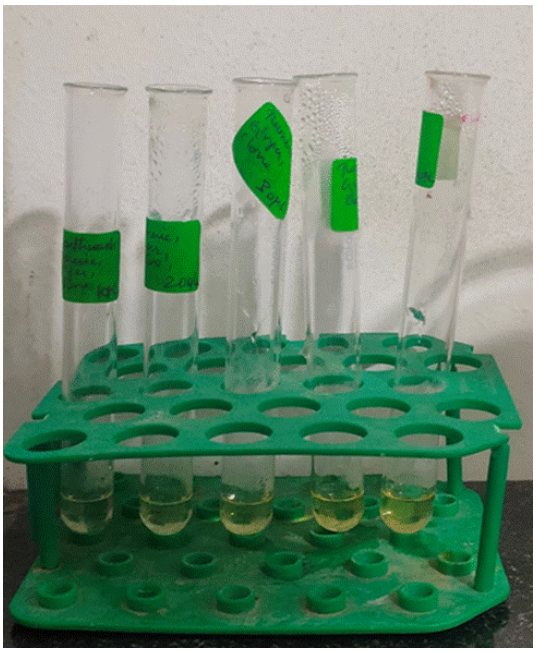

FIGURE 2. Anti-inflammatory activity of novel polyherbal mouthwash and commercial mouthwash at different concentrations.

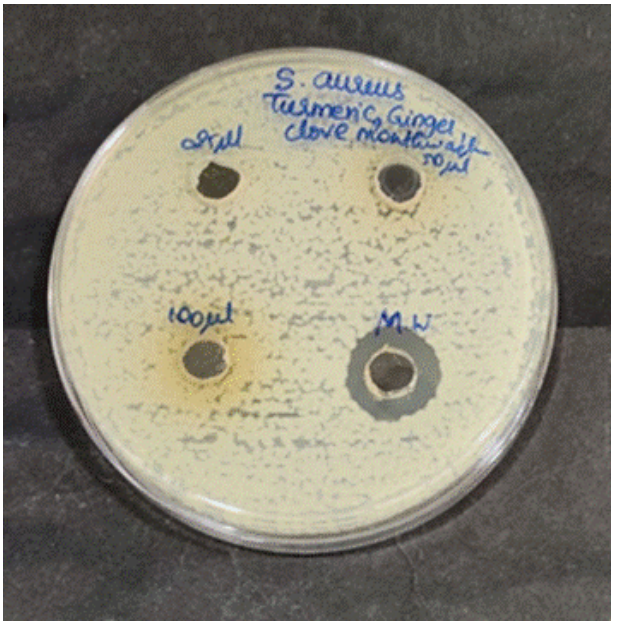

FIGURE 3. Antimicrobial activity of novel polyherbal mouthwash and commercial mouthwash at different concentrations against *Staphylococcus aureus*.

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FIGURE 4. Antimicrobial activity of novel polyherbal mouthwash and commercial mouthwash at different concentrations against *Candida albicans*.

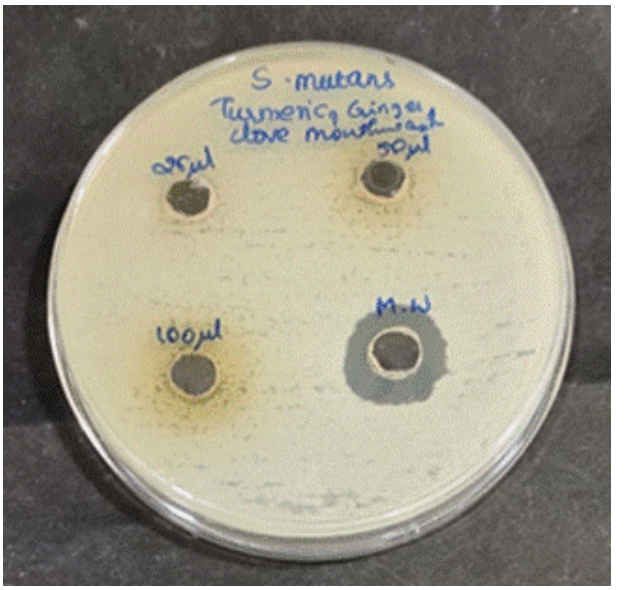

FIGURE 6. Antimicrobial activity of novel polyherbal mouthwash and commercial mouthwash at different concentrations against *Streptococcus mutans*.

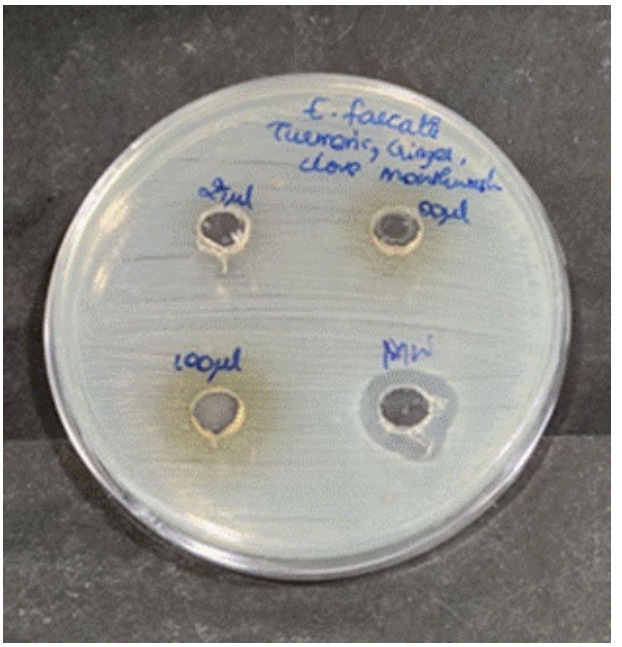

FIGURE 5. Antimicrobial activity of novel polyherbal mouthwash and commercial mouthwash at different concentrations against *Enterococcus faecalis*.

organisms except *E. faecalis* had an equal zone of inhibition. The commercial herbal mouthwash (Hiora) had greater antibacterial effect on all organisms, highest being with *E. faecalis* (22 mm) at all concentrations. However, there is a decent amount of antimicrobial activity of this novel mouthwash against all organisms, especially *E. faecalis* (18 mm) at 100 µL (Figure 1) (Graph 1).

Anti-inflammatory activity

The results indicated that the percentage of inhibition was almost equal to that of the standard herbal mouthwash (Hiora). It was highest (56%) at 20 µL concentration, followed by 30 µL (68%), and least at 40 µL (71%) when compared to the commercial mouthwash (Table 2). However, there was not much difference between both the mouthwashes. Our novel mouthwash showed a decent anti-inflammatory activity compared to the commercial mouthwash at 20 µL (Figure 2) (Graph 2).

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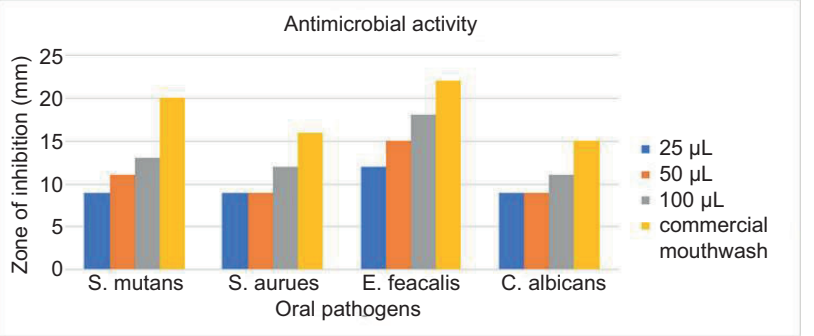
TABLE 1. Antimicrobial activity of novel polyherbal mouthwash and commercial mouthwash at different concentrations.

Microbes	25 µL	50 µL	100 µL	Commercial mouthwash
Streptococcus mutans	9	11	13	20
Staphylococcus aureus	9	9	12	16
Enterococcus faecalis	12	15	18	22
Candida albicans	9	9	11	15



GRAPH 1. The above graph represents the Anti-microbial activity of novel polyherbal mouthwash and commercial mouthwash at different concentrations against *Streptococcus. mutans*, *Staphylococcus. aureus*, *Enterococcus. faecalis*, and *Candida albicans*.

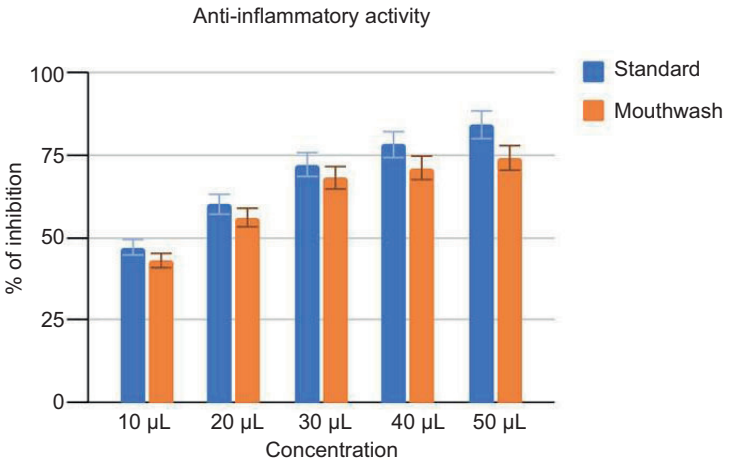
TABLE 2. Anti-inflammatory activity of novel polyherbal mouthwash and commercial mouthwash at different concentrations.

Polyherbal mouthwash	Standard	Mouthwash
10 µL	47	43
20 µL	60	56
30 µL	72	68
40 µL	78	71
50 µL	84	74

DISCUSSION

India is a land filled with nature's medicinal plants.[27] Herbal extracts are known to possess antimicrobial compounds, especially against bacterial pathogens such as *S. mutans*, which is considered the main culprit among cariogenic microorganisms. The aim of this study was to prepare a polyherbal mouthwash and evaluate its antimicrobial and anti-inflammatory efficacy against a commercially available herbal mouthwash. The primary outcomes of this study were to assess the antimicrobial and anti-inflammatory properties of novel herbal mouthwash against common oral flora.

The novel formulation of herbal mouthwash, with the use of various herbal extracts to enhance the benefits of the combination of multiple herbs, was the strength of the study. Several studies have been published which compare the efficacy of herbal extracts as mouthwashes, either individually or in combinations, to CHX. The herbal mixture used in this study is one-of-a-kind and, to the best of our knowledge, the first of its kind in combination. Using an antimicrobial agent as a mouthrinse might inhibit the growth of microorganisms, thus preventing the development of dental caries. Fluoride and CHX are two commonly used anticariogenic oral rinses. The herbal preparations are moderate in efficacy and less toxic than the commonly used pharmaceutical mouthwashes.[29]



GRAPH 2. The above graph represents the Anti-inflammatory activity showing percentage of inhibition of novel polyherbal mouthwash and commercial mouthwash at different concentrations.

As this is a novel combination, there are no previous studies comparing this combination, but previous studies on the herbs individually had shown significant differences in the outcome parameters.

The results indicated that the antimicrobial effect of the mouthwash was effective in inhibiting the growth of *S. aureus, S. mutans, E. faecalis,* and *C. albicans*. At 25, 50, and 100 µL, *E. faecalis* had the highest zone of inhibition compared to the other three microorganisms. At 50 µL, *S. mutans* (11 mm) had a greater zone of inhibition than *S. aureus* (9 mm) and *C. albicans* (9 mm) (Table 1, Figure 1). At 25 µL, all the three organisms except *E. faecalis* had an equal zone of inhibition. The commercial herbal mouthwash (Hiora) had greater antibacterial effect on all organisms, highest being with *E. faecalis* (22 mm) at all concentrations. However, there is a decent amount of antimicrobial activity of this novel mouthwash against all organisms especially *E. faecalis* (18 mm) at 100 µL.

The anti-inflammatory results indicated that the percentage of inhibition was almost equal to that of the standard herbal mouthwash (Hiora). It was highest (56%) at 20 µL concentration, followed by 30 µL (68%), and least at 40 µL (71%) when compared to commercial mouthwash (Table 2, Figure 1). However, there was not much difference between both the mouthwashes. Our novel mouthwash showed a decent anti-inflammatory activity compared to the commercial mouthwash at 20 µL.

In this study, the ingredients used in the formulated herbal mouthwash were chosen after a thorough understanding of their pharmacological actions, to optimize the combination. The herbal blend's antibacterial effect could be attributed to the combined effect of each individual component. Turmeric's anti-inflammatory, antimicrobial, antioxidant, immunostimulant, and antiseptic properties are well known. The anti-inflammatory action is due to its selective inhibitory action of prostaglandin E2 synthesis and thromboxane and inflammatory mediators of arachidonic acid metabolism.[28,29] As the molecules are lipophilic in nature, they cause rapid permeability of the cell membrane as in the process of apoptosis and induce change in the structure and integrity of the bacterial cells.[30] *Z. officinale* is a medicinal plant that may reduce the biosynthesis of prostaglandins[31] through the inhibition of cyclooxygenases 1 and 2,[31] and shows antibacterial properties[32] against a large number of

microorganisms, including *S. mutans*.[32] *Z. officinale* is currently one of the most widely used plants in the Indian system of traditional medicine. Some studies have revealed its numerous pharmacologic activities, such as antioxidant, antibacterial, and anti-inflammatory properties.[32,33] Clove is a natural antibiotic with broad antibacterial, antifungal, and antiviral activities.[20] Dentists use it in bandages for minor wounds and as an analgesic in the treatment of painful and infective oral disorders. It is also used in medical and dental professions, as an analgesic, antispasmodic, and general antiseptic.[21]

All the studies cited in this article are mostly compared to CHX, whereas our study results were compared with commercially available herbal mouthwash (Hiora); hence, this combination can be much beneficial when compared to other chemical mouthwashes.

Combining several such herbal extracts, as in this study, would undoubtedly result in mouthwash with a wide range of benefits in terms of reducing pathogenic oral microorganisms. Because the benefits of mouthwashes are better delivered over time, switching to herbal mix mouthwashes is preferable because they are traditionally and culturally acceptable, economically feasible, safe, and efficient.

Further research regarding new herbal combinations as mouthwashes on oral pathogenic organisms in various clinical conditions is required to implicate the benefits of herbal extracts over commercially available mouthwashes.

CONCLUSION

Our study showed no difference between the novel mouthwash and commercially available herbal mouthwash. Novel mouthwash showed similar, comparable anti-inflammatory effect to commercial mouthwash but did not show any such effects in terms of the antimicrobial effect. Because this combination is readily available, it can be a cost-effective alternative to commercially available herbal mouthwash.

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