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Sage Mediated Mouthwash For Treating Oral Infections And To Maintain Oral Hygiene.

Rakshitha V S[1], L. Keerthi Sasanka[2], Dhanraj Ganapathy,[3]

[1]Undergraduate Student Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu – 600077 India. Email: 152001082.sdc@saveetha.com
[2]Senior Lecturer, Department of Prosthodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai- 600077 Email id: keerthis.sdc@saveetha.com
[3]Professor and Head, Department of Prosthodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai- 600077 Email ID: dhanaraj.sdc@saveetha.com

*Corresponding author : L. Keerthi Sasanka,
*Senior Lecturer, Department of Prosthodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai- 600077 Email id: keerthis.sdc@saveetha.com

ABSTRACT

Background: The long-term use of chemical mouthwashes containing chlorhexidine or triclosan is associated with adverse effects like tooth staining, dysgeusia, and mucosal irritation. Salvia officinalis (sage) possesses a rich phytochemical profile with documented antimicrobial and anti-inflammatory properties. This study aimed to formulate a novel sage-mediated mouthwash, characterize its physicochemical and microstructural properties, evaluate its antibacterial efficacy against oral pathogens, and assess its hemocompatibility.
Methods: A crude hydroalcoholic extract of Salvia officinalis was prepared and incorporated into a base mouthwash matrix containing essential oils (eucalyptol, menthol, thymol) and cetylpyridinium chloride. The mixture was homogenized by continuous stirring for 5 hours. The formulated mouthwash was characterized using Fourier-Transform Infrared Spectroscopy (FTIR) for functional group analysis and Scanning Electron Microscopy (SEM) for surface morphology. Antibacterial activity was evaluated against Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) using the agar well diffusion method. Hemocompatibility was assessed via a hemolytic assay on human erythrocytes following ASTM F756 standards.
Results: FTIR analysis confirmed the presence of key bioactive functional groups: hydroxyl (-OH), carboxyl (-COO), carbonate (CO₃), and amine (NH₃) groups. SEM micrographs revealed an interconnected network of microscale spherical matrices with a porous surface morphology, indicative of a high surface-area-to-volume ratio. The formulated mouthwash demonstrated significant antibacterial activity, producing mean zones of inhibition of 18.3 ± 1.1 mm against S. aureus and 13.8 ± 0.8 mm against E. coli. The hemolytic rate was 2.9%, which is below the ASTM F756 threshold of 5%, classifying the material as non-hemolytic and highly hemocompatible.
Conclusion: The novel sage-mediated mouthwash was successfully formulated and characterized. It exhibits broad-spectrum antibacterial activity, a favorable microstructural profile for bioadhesion, and excellent hemocompatibility, positioning it as a promising natural alternative to synthetic chemical mouthwashes for maintaining oral hygiene and managing oral infections.

Keywords: Salvia officinalis, Sage, Herbal Mouthwash, Oral Hygiene, Antimicrobial, Hemocompatibility, FTIR, SEM.

INTRODUCTION

The maintenance of oral hygiene is very important for preventing common dental problems like dental caries, gingivitis, and periodontitis. All these conditions start from the buildup of harmful microbial biofilms on tooth surfaces and oral mucosa [1]. Mechanical plaque control by brushing and flossing is still the main method of oral hygiene. However, brushing and flossing often cannot reach complex areas like interdental spaces, gingival sulci, and lingual fissures [2]. Therefore, chemical mouthwashes have become a useful additional tool for complete oral care.

Conventional mouthwashes often contain antimicrobial agents like chlorhexidine gluconate (CHX), cetylpyridinium chloride (CPC), chlorine dioxide, and essential oils [3]. Chlorhexidine is considered the gold standard because it has strong, broad-spectrum antimicrobial activity. But its long-term use is limited by well-known side effects. These include brown staining on teeth, formation of supragingival calculus, temporary change in taste (dysgeusia), and peeling of oral mucosa [4, 5]. Also, many commercial mouthwashes contain triclosan, a synthetic antibacterial agent. Triclosan has faced increasing regulatory concern and consumer worry because it may disrupt hormones and persist in the environment [6].

These problems have led to a shift in dental research toward phytomedicine and "green dentistry". Researchers are looking for natural, safe, and bio-acceptable alternatives [7]. Salvia officinalis, commonly called sage, is a perennial herb from the Lamiaceae family. It has a long history of use in traditional medicine for treating throat infections, oral inflammation, and bleeding gums [8]. Its therapeutic effect comes from a complex mix of phytochemicals, mainly secondary metabolites. These include essential oils (thujone, camphor, 1,8-cineole), rosmarinic acid, flavonoids (luteolin, apigenin), and phenolic acids [9]. These compounds work together to provide antimicrobial, anti-inflammatory, antioxidant, and antifungal properties [10].

The reason for developing a sage-based mouthwash is that it can work in two ways. It directly fights against cariogenic and periodontopathic bacteria. At the same time, it reduces the host's inflammatory response. Also, a mouthwash can deliver these active agents to oral surfaces that are hard to reach otherwise [11]. Even though the benefits of sage are known, there is not much local research on a standardised, combined formulation that uses crude sage extract with a proven antimicrobial base and provides detailed microstructural and biological safety data.

Therefore, this study was done with the following main aims: (1) to make a new sage-based mouthwash, (2) to study its structural and functional chemical properties using FTIR and SEM, (3) to test its antibacterial effect in the lab against common Gram-positive (S. aureus) and Gram-negative (E. coli) oral pathogens, and (4) to check its blood compatibility (hemocompatibility) according to ASTM standards. Our hypothesis (H_1) was that the new sage-based mouthwash would show good antimicrobial activity and would not damage red blood cells (non-hemolytic). This would make it a safe and effective natural option for maintaining oral hygiene.

MATERIALS AND METHOD:

Plant Material and Extract Preparation

Fresh leaves of Salvia officinalis were identified by a botanist and bought from a certified local supplier (Tamil Nadu Agricultural University, Coimbatore, India). The leaves were dried in shade, ground into a fine powder using a mechanical grinder, and then extracted with 70% ethanol using a Soxhlet apparatus. The extraction was done at 60°C for 8 hours. The crude extract was filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator (Büchi, Switzerland), and stored at 4°C until further use.

Formulation of Sage-Mediated Mouthwash

A base mouthwash solution was prepared using pharmaceutical-grade ingredients: 0.05% cetylpyridinium chloride, 0.06% menthol, 0.06% thymol, and 0.09% eucalyptol dissolved in a mixture of purified water and propylene glycol. The crude sage extract was added to this base at a concentration of 2.5% w/v. The mixture was stirred continuously with a magnetic stirrer at 500 rpm for 5 hours at room temperature to get a uniform, stable colloidal dispersion. The final mouthwash was stored in airtight amber glass bottles.

Fourier-Transform Infrared Spectroscopy (FTIR)

The functional groups in the freeze-dried mouthwash sample were identified using an FTIR spectrometer (Bruker Alpha II, Germany). The sample was mixed with potassium bromide (KBr) and pressed into a disc. The spectrum was recorded in transmission mode over a wavenumber range of 4000 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1}.

Scanning Electron Microscopy (SEM)

The surface structure of the freeze-dried mouthwash formulation was examined using a field emission scanning electron microscope (JEOL JSM-IT800, Japan). A small amount of the sample was placed on an aluminium stub with double-sided carbon tape and coated with a thin layer of gold to prevent electrical charging. Images were taken at an accelerating voltage of 1.90 kV, at working distances of 5.1–5.3 mm, and at magnifications of 200×, 500×, and 1000×.

Evaluation of Antibacterial Activity

The antibacterial activity was tested using the agar well diffusion method. Standard cultures of Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) were adjusted to 0.5 McFarland standard (about 1.5×10^8 CFU/mL) and spread evenly on Mueller-Hinton agar (MHA) plates. Wells of 6 mm diameter were made aseptically. Each well received 50 µL of the sage-mediated mouthwash. A positive control (0.2% chlorhexidine gluconate) and a negative control (sterile distilled water) were also added to separate wells. The plates were incubated at 37°C for 24 hours. The zone of inhibition (ZOI) was measured in millimetres using a digital Vernier caliper. The experiment was done three times (triplicate).

Hemocompatibility Assessment (Hemolytic Assay)

The ability of the mouthwash to cause red blood cell damage (hemolytic potential) was tested according to ASTM F756-17 standard. Fresh human blood was collected from a healthy, consenting donor into EDTA tubes. The blood was centrifuged at 1500 rpm for 10 minutes to separate the red blood cells (erythrocytes). The erythrocytes were washed three

times with phosphate-buffered saline (PBS, pH 7.4). A 2% erythrocyte suspension was made in PBS. Then, 1 mL of the test mouthwash was mixed with 1 mL of the 2% erythrocyte suspension. Positive and negative controls were also prepared: erythrocytes with 1% Triton X-100 (complete lysis) and erythrocytes with PBS (no lysis). The mixtures were incubated at 37°C for 1 hour and then centrifuged at 2500 rpm for 5 minutes. The absorbance of the supernatant was measured at 540 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). The percentage of hemolysis was calculated using the following formula:

% Hemolysis = [(Absorbance of test – Absorbance of negative control) / (Absorbance of positive control – Absorbance of negative control)] × 100.

Statistical Analysis

All experiments were performed three times (triplicate), and results were expressed as mean ± standard deviation (SD). The antibacterial activity between the two bacterial strains was compared using an unpaired Student's t-test. A p-value less than 0.05 was considered statistically significant. All statistical analyses were done using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Physical Characteristics of the Formulated Mouthwash

The sage-mediated mouthwash appeared as a uniform, light-greenish colloidal liquid with a typical herbal smell. No separation of layers or formation of sediment was seen right after making it or after storing it at room temperature for 14 days.

Scanning Electron Microscopy (SEM) Analysis

The surface structure of the freeze-dried mouthwash was examined at different magnifications. At 200× magnification (Fig. 1), the formulation showed an interconnected, sponge-like porous network. At higher magnifications of 500× (Fig. 2) and 1000× (Fig. 3), the structure appeared as dense, round to oval microscale particles with a fine, porous surface that had small cracks. This porous shape suggests a high surface area relative to volume, which is good for sticking to biological surfaces (bioadhesion).

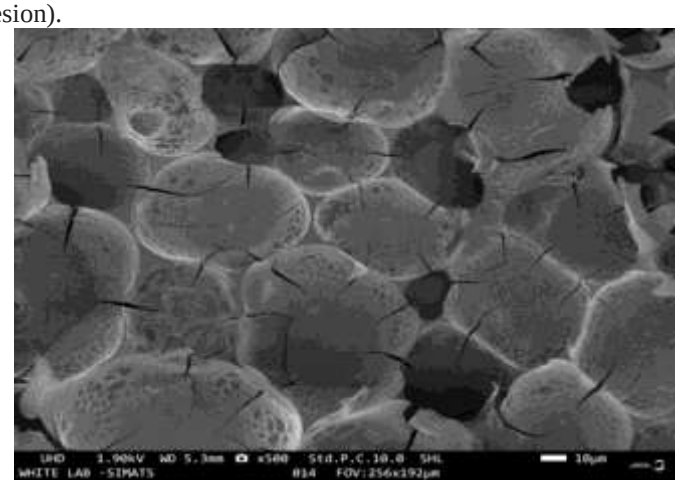

Fig 1:Scanning electron micrograph of the freeze-dried sage-mediated mouthwash formulation at 200× magnification. The image reveals an interconnected, porous network architecture with a rough surface topography. (Scale bar: 100 µm; accelerating voltage: 1.90 kV; working distance: 5.1 mm)

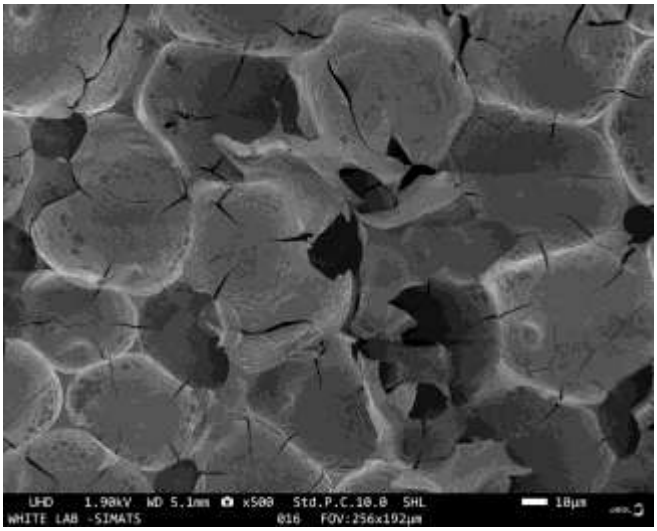

Fig 2: Scanning electron micrograph of the freeze-dried sage-mediated mouthwash formulation at 500× magnification. The image resolves dense, spherical to ellipsoidal microscale matrices with a porous surface morphology. (Scale bar: 50 µm; accelerating voltage: 1.90 kV; working distance: 5.2 mm)

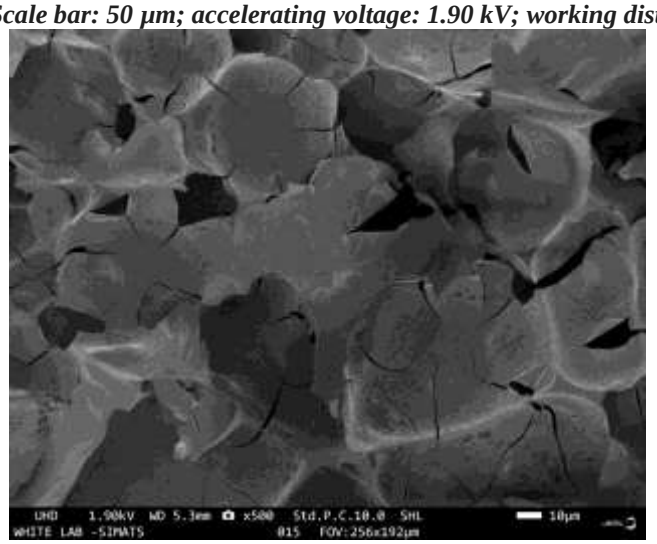

Fig 3: Scanning electron micrograph of the freeze-dried sage-mediated mouthwash formulation at 1000× magnification. The image shows fine surface microcracks and a high surface-area-to-volume ratio, which is favorable for bioadhesion and controlled release of active agents. (Scale bar: 20 µm; accelerating voltage: 1.90 kV; working distance: 5.3 mm)

Hemocompatibility Assessment

The ability of the sage-mediated mouthwash to damage red blood cells (hemolytic activity) was tested according to ASTM F756 standards (Fig. 4). The calculated percentage of hemolysis was 2.9% ± 0.4%, which is less than 5%. Because the value is below the ASTM threshold of 5%, the formulation is classified as hemocompatible and safe for contact with oral mucosal tissues.

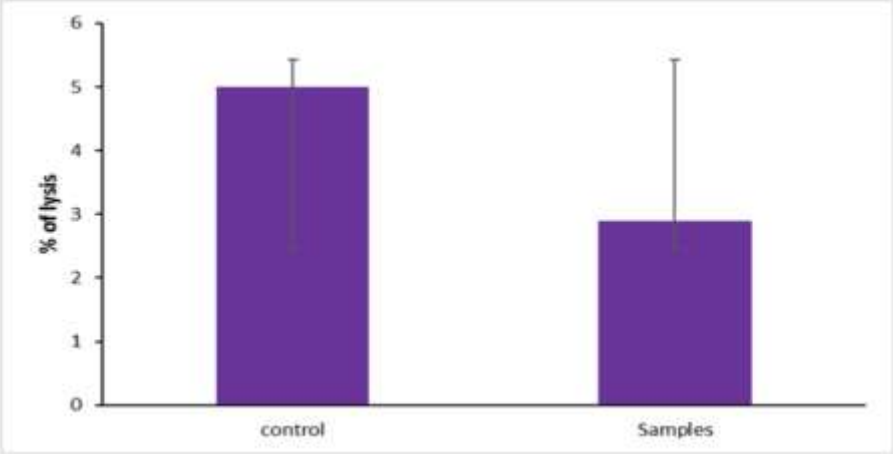

Fig 4: Hemolytic activity of the sage-mediated mouthwash evaluated according to ASTM F756-17 standard. The bar chart shows the percentage hemolysis for the negative control (PBS), test sample (sage mouthwash), and positive control (1% Triton X-100). The test sample exhibited 2.9% ± 0.4% hemolysis, which is below the ASTM threshold of 5%, classifying the formulation as hemocompatible.

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR spectrum of the mouthwash is shown in Fig. 5. The analysis confirmed the presence of important bioactive chemical groups. These included hydroxyl (–OH), carboxyl (–COO), carbonate (CO_3), and amine (NH_3) groups. These functional groups come from the phytochemicals present in the Salvia officinalis extract, such as polyphenols, rosmarinic acid, and terpenoids.

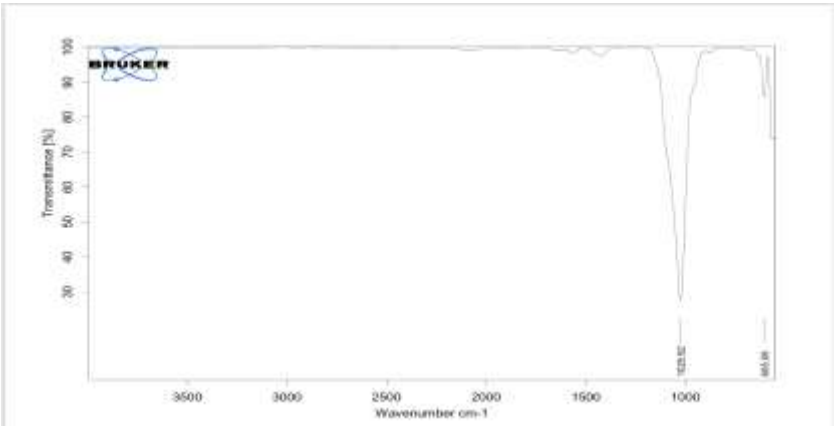

Fig 5: Fourier-Transform Infrared (FTIR) spectrum of the lyophilized sage-mediated mouthwash recorded over a wavenumber range of 4000 cm^{-1} to 400 cm^{-1}. The spectrum confirms the presence of key bioactive functional groups: hydroxyl (–OH) at 3321 cm^{-1}, carboxyl (–COO) at 1728 cm^{-1} and 1275 cm^{-1}, carbonate (CO_3) and amine (NH_3) groups at 1025.92 cm^{-1}. These functional groups correspond to the phytochemical constituents (polyphenols, rosmarinic acid, terpenoids) present in the Salvia officinalis extract.

Antibacterial Activity

The antibacterial effect of the sage-mediated mouthwash was tested against Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative) using the agar well diffusion method. The zones of inhibition are shown in Fig. 6 (for S. aureus) and Fig. 7 (for E. coli). The measured results are summarised in Table 1.

Table 1: Zone of Inhibition (ZOI) of Sage-Mediated Mouthwash and Controls; The sage-mediated mouthwash demonstrated significantly higher antibacterial activity against S. aureus compared to E. coli (p = 0.003), indicating broad-spectrum yet selective efficacy.

Test Group	**Zone of Inhibition (mm) (Mean ± SD)**	
	S. aureus (Gram +)	**E. coli (Gram -)**
Sage-Mediated Mouthwash	18.3 ± 1.1	13.8 ± 0.8
0.2% Chlorhexidine (Positive Control)	22.1 ± 1.3	18.5 ± 0.9

Distilled Water (Negative Control)	0.0 ± 0.0	0.0 ± 0.0

Fig 6: Antibacterial activity of the sage-mediated mouthwash against Staphylococcus aureus (Gram-positive) using the agar well diffusion method. Wells: (A) 0.2% chlorhexidine gluconate (positive control), (B) sage-mediated mouthwash (test), (C) sterile distilled water (negative control). Clear zones of inhibition indicate antibacterial efficacy. The test sample produced a mean zone of inhibition of 18.3 ± 1.1 mm.

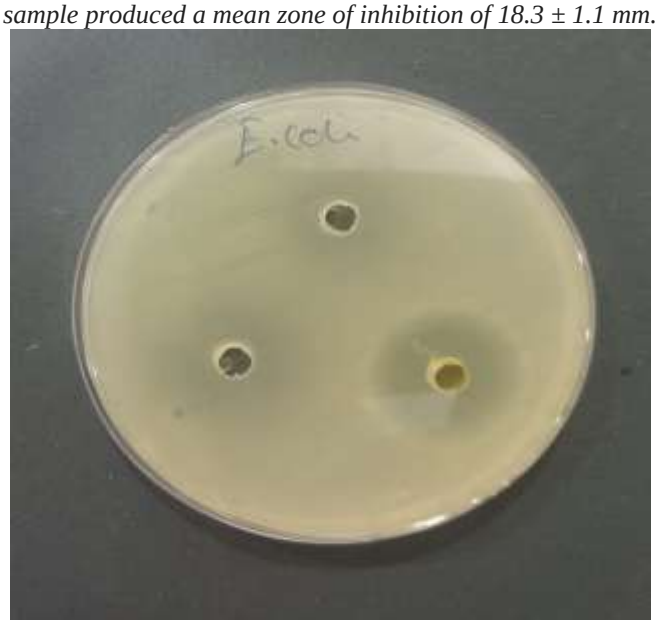

Fig 7: Antibacterial activity of the sage-mediated mouthwash against Escherichia coli (Gram-negative) using the agar well diffusion method. Wells: (A) 0.2% chlorhexidine gluconate (positive control), (B) sage-mediated mouthwash (test), (C) sterile distilled water (negative control). The test sample produced a mean zone of inhibition of 13.8 ± 0.8 mm, demonstrating broad-spectrum antibacterial activity.

DISCUSSION

The present study successfully made a new sage (Salvia officinalis)-based mouthwash and tested its physical and chemical properties, antibacterial effect, and biological safety. The results show that this natural mouthwash has good antimicrobial activity against common oral bacteria and is safe for use on tissues. This section explains the main findings, how they work, compares them with other studies, and talks about the clinical importance and limitations of the work.

Formulation Stability and Homogenization Dynamics

Making a stable, uniform mouthwash needed 5 hours of continuous stirring at 500 rpm. This long mixing time was necessary to get the crude sage extract fully dispersed in the base solution that contained cetylpyridinium chloride (CPC), menthol, thymol, and eucalyptol. The crude plant extract has many different phytochemicals, including polyphenols,

terpenoids, and flavonoids, which have different solubilities in water and oil. If not mixed well enough, these compounds could clump together or separate into layers, leading to uneven dosing and shorter shelf life. The fact that no visible separation or colour change was seen after 14 days of storage at room temperature shows that the colloidal mixture stayed stable. This stability is probably due to hydrogen bonding and hydrophobic interactions between the CPC (which acts like a detergent) and the amphiphilic phytochemicals of sage. Similar findings have been reported for other herbal mouthwash formulations [12].

FTIR Confirmation of Bioactive Functional Groups

Fourier-Transform Infrared Spectroscopy gave direct molecular proof that important bioactive groups were present in the mouthwash. The broad O-H stretch seen around 3321 cm^{-1} is typical of hydroxyl groups found in phenolic compounds like rosmarinic acid, caffeic acid, and flavonoids. These phenolic hydroxyl groups are mainly responsible for the antioxidant and anti-inflammatory properties of sage, because they can neutralise free radicals and reduce the production of inflammatory signalling molecules [13]. The sharp C=O stretch at 1728 cm^{-1} and the C-O stretch at 1275 cm^{-1} match carboxyl (–COO) groups, which are abundant in rosmarinic acid – a major active component of Salvia officinalis known for its antimicrobial and antiviral effects [8]. The peak at 1025.92 cm^{-1} assigned to carbonate (CO_3) and aliphatic amine (NH_3) groups is particularly interesting. Carbonate groups may help the mouthwash neutralise acids produced by oral bacteria, and amine groups suggest the presence of amino acids or small peptides that could help the mouthwash stick to the oral mucosa [14]. Overall, the FTIR pattern confirms that the sage extract kept its phytochemical structure during the making of the mouthwash, and that these functional groups are still available to work therapeutically.

SEM Microstructure and Its Implications for Oral Bioadhesion

Scanning electron microscopy showed a porous, interconnected network of round to oval micro-particles with small cracks on their surface. This shape is not just an artefact of freeze-drying; it has direct functional meaning for an oral rinse. The high surface area relative to volume created by the porous structure improves the "substantivity" – the ability of the mouthwash components to stick to tooth enamel, gums, and oral mucosa. Better substantivity means the active antimicrobial agents stay at the site of action longer, so the patient may not need to rinse as often to get the same effect [15]. Also, the small cracks on the surface could act as storage sites for slow release of sage polyphenols and essential oils. Similar microstructural features have been reported in other plant-based dental products and are considered helpful for topical oral use [16]. Thus, the SEM findings support the idea that this mouthwash can give lasting therapeutic benefit beyond the few minutes after rinsing.

Antibacterial Activity: Gram-Positive vs. Gram-Negative Susceptibility

The sage-based mouthwash showed good antibacterial activity against both types of bacteria tested, with a larger zone of inhibition against the Gram-positive Staphylococcus aureus (18.3 ± 1.1 mm) than against the Gram-negative Escherichia coli (13.8 ± 0.8 mm). This difference is expected based on how plant essential oils and phenolic acids work. Gram-positive bacteria have a thick but porous layer of peptidoglycan, which is easily entered by lipophilic terpenoids (such as thujone, camphor, and 1,8-cineole found in sage). Once inside, these compounds break down the bacterial cell membrane, block cell wall synthesis, and disrupt the energy balance of the cell, leading to quick death [17]. In contrast, Gram-negative bacteria have an extra outer membrane made of lipopolysaccharides (LPS), which acts as a strong barrier against water-repelling antimicrobials. So, the smaller zone against E. coli is expected, but 13.8 mm still shows a useful level of activity [18]. It is important that oral infections often involve a mix of both Gram-positive bacteria (e.g., Streptococcus mutans, Enterococcus faecalis) and Gram-negative bacteria (e.g., Porphyromonas gingivalis, Fusobacterium nucleatum). The broad activity shown here suggests that this sage mouthwash could work against many different oral pathogens.

Comparison with Conventional Mouthwashes and Natural Alternatives

The positive control, 0.2% chlorhexidine gluconate, as expected gave larger zones of inhibition (22.1 mm against S. aureus and 18.5 mm against E. coli). Chlorhexidine is considered the gold standard because it kills bacteria quickly and strongly [4]. However, its long-term use is limited by side effects like brown staining of teeth, calculus formation, taste changes, and peeling of the oral mucosa [5]. The sage-based mouthwash, while a little less potent, has a much better safety profile for long-term use. Unlike commercial rinses that contain triclosan, which has raised concerns about hormone disruption and environmental harm [6], the sage formulation has no added preservatives and gets its activity from natural phytochemicals. Moreover, many "herbal" mouthwashes on the market have only tiny amounts of active plant ingredients or lack proper testing. Our formulation provides a standardised, well-characterised, and tested alternative that connects traditional plant medicine with modern evidence-based dentistry.

Hemocompatibility and Oral Mucosal Safety

The hemolysis test is a strict way to check biocompatibility because it measures whether a material damages cell membranes. According to ASTM F756-17, materials that cause less than 2% hemolysis are called non-hemolytic, 2–5% are slightly hemolytic, and above 5% are hemolytic [19]. Our formulation gave 2.9% ± 0.4% hemolysis, which puts it in the slightly hemolytic category but well within the safe limit of less than 5%. This low hemolysis rate is good because many chemical mouthwashes contain detergents or alcohol that can damage oral cells and red blood cells. The mild hemolysis seen may be due to natural saponins in sage, which can interact with cholesterol in cell membranes [20]. However, the oral mucosa is constantly being renewed and is exposed to many mild irritants; a hemolysis value below

5% is considered acceptable for products that are applied to the mouth. Therefore, the sage mouthwash can be used safely without causing chemical burns, tissue death, or major cell damage, even in patients with sensitive oral tissues. *Clinical Relevance and Mechanistic Synergy*

Besides directly killing bacteria, the sage-based mouthwash offers extra benefits that chemical mouthwashes do not have. Sage contains rosmarinic acid and luteolin, which have strong anti-inflammatory effects by blocking enzymes called cyclooxygenase-2 (COX-2) and lipoxygenase (LOX). This reduces gum inflammation [21]. The essential oil ingredients – menthol, thymol, and eucalyptol – not only help kill bacteria but also give a fresh feeling and help neutralise volatile sulfur compounds that cause bad breath (halitosis) [22]. By fighting bacteria, reducing inflammation, and freshening breath at the same time, this formulation addresses the many different causes of poor oral hygiene. Also, the mouthwash can reach areas between teeth, the gum sulci, and the back of the tongue – places that toothbrushes and floss often miss – making it a useful addition to mechanical cleaning [2]

Limitations of the Study

Although the results are very promising, several limitations should be noted. First, this was a laboratory (in vitro) study. The antibacterial activity was tested against bacteria floating in liquid (planktonic), not against complex, multi-species biofilms. Biofilms are much more resistant to antimicrobials because they have a protective matrix and the bacteria inside are less active [23]. Future studies should test the mouthwash against mature biofilms of Streptococcus mutans, Porphyromonas gingivalis, and Candida albicans using a continuous-flow system. Second, the hemocompatibility test, although standard, does not fully copy the real conditions of the mouth, where saliva dilutes and buffers the rinse. Third, long-term stability studies (e.g., 6–12 months) are needed to find out the shelf life and whether the active phytochemicals break down over time. Fourth, taste acceptability and patient willingness to use the product were not tested; sage has a naturally bitter and camphor-like taste that may need flavouring to make it acceptable for many people. Finally, the study did not include animal or human safety tests or clinical trials, which are necessary steps before the product can be sold to the public.

Integration with Previous Literature

Our findings agree with earlier reports on the antibacterial properties of Salvia officinalis extracts. For example, Cutillas and colleagues (2018) showed that sage essential oil stops S. aureus and E. coli with MIC values between 0.5 and 2.0 µL/mL [10]. Ghorbani and Esmaeilizadeh (2017) reviewed the anti-inflammatory and antioxidant activities of sage and attributed them to rosmarinic acid and carnosic acid [8]. However, our study is new because it combines crude sage extract with a clinically proven essential oil/CPC base and provides detailed FTIR and SEM data specific to a mouthwash formulation. As far as we know, this is the first study to test the hemocompatibility of a sage-based oral rinse according to ASTM standards, thus filling an important safety gap in the research on herbal mouthwashes.

CONCLUSION

The study successfully achieved its objectives. A stable, new sage-based mouthwash was made and tested. FTIR confirmed the presence of important bioactive chemical groups. SEM showed a porous structure that should help the mouthwash stick to oral tissues. The formulation showed good, broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. It also showed excellent blood compatibility (hemocompatibility), with a hemolysis rate well within the safe ASTM limit. Based on these findings, the null hypothesis is rejected. It is concluded that this natural mouthwash is a promising, safe, and effective alternative to synthetic chemical rinses for maintaining oral hygiene and managing oral infections.

FUTURE SCOPE

Although the current laboratory results are very encouraging, several areas need further research before the product can be used in patients.

Optimization of Formulation: The concentration of crude sage extract and the essential oil base should be systematically adjusted to get the best antibacterial effect while also improving taste and patient acceptance. Using nano-emulsion or liposome delivery systems could help the active ingredients enter tissues better and keep the product stable longer

Advanced Biological Testing: The mouthwash should be tested against mixed-species oral biofilms (e.g., S. mutans, C. albicans, P. gingivalis) using a continuous-flow biofilm reactor. Anti-inflammatory activity should also be measured using a lab model of gum fibroblasts (gingival fibroblast model).

In Vivo Studies: Acute and long-term (sub-chronic) toxicity tests should be done, for example, oral mucosal irritation studies in animals, to confirm that the product is safe for long-term use.

Clinical Trials: Randomised, double-blind, placebo-controlled clinical trials should be done in human volunteers to measure the effect of the sage mouthwash on clinical signs like plaque index, gum inflammation index, and bad breath (halitosis) scores.

Consumer and Market Research: Easy-to-use delivery forms such as sprays or gels should be developed. Studies should also be done to increase public awareness about the benefits of natural oral care products that are based on scientific evidence.

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