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Anti-inflammatory activity of *Salvadora persica* and *Andrographis paniculata*formulation-based mouthwash

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Abstract:
Biofilm formation and bacterial growth are crucial for the retention of oral health. Several plants like *Salvadora persica* also known as miswak and *Andrographis paniculata*have been used for oral hygiene since ancient times. Newer research suggests better for their extracts in which both types manage to present high bactericidal and anti-biofilm efficiency. The objective of this study was to prepare and characterize a new oral-herbal mouthwash formula that can be beneficial on bacteriostatic, biofilm breakdown & inflammation in the context of avoiding adverse effects. The mouthwash formulation containing the plant extracts were evaluated for their efficacy through different assays. This entailed the examination of their antibacterial potential towards typical oral pathogens and biofilm inhibitory properties. Further, the denaturation activity was studied using Bovine Serum Albumin (BSA) and Egg Albumin (EA) protein by employing Protein Denaturation constants with little unintended side effects of dual cavity-binding.
Key words: *Salvadora persica*,*Andrographis paniculata*,inflammation,anti - biofilm, egg albumin.

1 Introduction

In recent years, there has been a growing interest in herbal medicine as an unconventional form of treatment. This shift is largely due to the effectiveness of herbal remedies in treating a wide range of conditions, making them a popular alternative to traditional medical interventions(Nordin 2012, Ambika, Manojkumar et al. 2019). Among the many plants used in herbal medicine, *Salvadora persica* and *Andrographis paniculata*have gained significant attention for their potential health benefits, particularly in the realm of oral health.*Salvadora persica*, commonly known as miswak or siwak, originates from the toothbrush tree or Arak, which is native to West India and Africa. This small, light-yellow woody tree, also known as S. persica, is an evergreen perennial halophyte that thrives in harsh environments, including extremely saline soils and dry landscapes(Niazi, Naseem et al. 2016, Gandhi, Gurunathan et al. 2021). With a lifespan of 25 years, *S. persica* has a long history

of medicinal use among many ethnic communities, especially in Asia and Africa. Traditionally, *S. persica* has been used as a natural toothbrush, and its use has been endorsed for its numerous health benefits(Gandhi and Gurunathan 2022, Tayyeb, Priya et al. 2024).

One of the key properties of *S. persica* is its anti-inflammatory activity. Inflammation is a biological response of neurovascular tissues to adverse stimuli such as infections, damaged cells, and irritants(Farag, Abdel-Mageed et al. 2021, Sundaram, Bupesh et al. 2022). This response is essential for protecting the body by clearing and removing harmful stimuli, thereby initiating the healing process. Interestingly, *S. persica* has been found to possess significant anti-inflammatory properties. Research has shown that *S. persica* contains a diverse array of secondary metabolites that contribute to its medicinal properties, including anti-inflammatory, analgesic, and antioxidant effects. These properties support and validate the traditional use of *S. persica* in the treatment of oral diseases, highlighting its potential as a natural remedy for inflammation and other related conditions.Moreover, *Salvadora persica* is not just limited to anti-inflammatory properties; it also exhibits antibacterial and antifungal activities, making it effective against a broad spectrum of oral pathogens. The presence of compounds like salvadorine, chlorides, fluoride, silica, sulfur, and various alkaloids in *S. persica* contribute to its ability to inhibit the growth of bacteria and fungi, thus preventing dental caries and other oral infections. These properties make *S. persica* a comprehensive tool for maintaining oral hygiene and health(Almas and Almas 2013, Marunganathan, Kumar et al. 2024).

Andrographis paniculata, commonly referred to as the "King of Bitters," is another medicinal plant with a rich history of use in traditional medicine. Native to countries such as Thailand, Malaysia, Indonesia, China, Bangladesh, India, and the Philippines, A. paniculata has been utilized for centuries to treat a variety of illnesses. These include upper respiratory infections, cancer, diabetes, thrombosis, fever, gastrointestinal tract disorders, and herpes. Among its many benefits, A. paniculata is widely used in Ayurvedic medicine for its anti-inflammatory and antioxidant properties.The active compound in A. paniculata, known as andrographolide, has been extensively studied for its pharmacological properties(ABDULAZIZ, AL-MUFFTI et al. 2020, Velumani, Arasu et al. 2023). Andrographolide has demonstrated strong anti-inflammatory and antioxidant effects, which are believed to be the source of the plant's medicinal qualities. Research has shown that andrographolide influences various signaling pathways linked to inflammation, making it a promising candidate for the treatment of inflammatory lesions associated with a range of systemic disorders. The pharmacological characteristics of A. paniculata are well-documented, and its broad range of activities has led to its recognition as a contemporary catholicon—a universal remedy for a wide variety of ailments.Furthermore, *Andrographis paniculata*has been shown to possess immunomodulatory properties, enhancing the body’s immune response(Nivetha, Sakthi et al. 2020). This is particularly beneficial in combating oral pathogens and maintaining a healthy oral microbiome. The plant’s extracts have also been found to possess antiviral properties, adding another layer of protection against oral diseases that may be caused by viral infections.Given the well-documented anti-inflammatory and antimicrobial properties of both *Salvadora persica* and *Andrographis paniculata*, there is a compelling case for exploring their combined potential in a mouthwash formulation. This study aims to investigate the anti-inflammatory activity of a novel mouthwash that incorporates extracts from both plants. By combining the strengths of these two species, the formulation has the potential to offer a safe and effective natural solution for oral health.Periodontal disease, a common inflammatory condition affecting the gums and supporting structures of the teeth, can lead to severe dental issues if left untreated. Traditional treatments for periodontal disease often involve the use of antimicrobial agents and anti-inflammatory

medications, which can have side effects(Jain, Sayyed et al. 2020). A natural mouthwash formulation combining *Salvadora persica* and *Andrographis paniculata*could provide a holistic alternative, harnessing the anti-inflammatory and antimicrobial properties of these plants to reduce inflammation and promote oral health.In conclusion, the exploration of a mouthwash formulation based on *Salvadora persica* and *Andrographis paniculata*represents a promising avenue for enhancing oral health. The synergistic effects of these two plants could lead to a natural, effective, and safe alternative to conventional treatments for periodontal disease. This study will delve into the anti-inflammatory activity of the formulation, providing valuable insights into its potential benefits and efficacy in maintaining oral hygiene and preventing periodontal disease. Additionally, the use of such a formulation could significantly reduce the reliance on synthetic chemicals and drugs, promoting a more natural approach to oral care(Balaganesh, Leelavathi et al. 2021, Ponmanickam, Gowsalya et al. 2022).

2 Materials and method

2.1 Preparation of Plant Extracts

Salvadora persica and *Andrographis paniculata* were collected, cleaned, and dried in a shaded area to preserve their active compounds. The dried plant materials were ground into fine powders using a mechanical grinder. The powdered materials were subjected to solvent extraction using ethanol, a process that helps to efficiently isolate the bioactive components. The extracts were filtered to remove any particulate matter and concentrated using a rotary evaporator to remove the solvent, resulting in a dense, rich extract. The concentrated extracts were then stored in amber glass bottles at 4°C to prevent degradation and preserve their efficacy until further use. This meticulous preparation process ensures that the extracts retain their potent antibacterial, anti-biofilm, and anti-inflammatory properties, which are critical for the effectiveness of the herbal mouthwash formulation(Ozair and Ozair , Umapathy, Pan et al. 2024).

2.2 Formulation of Herbal Mouthwash

The mouthwash formulation was prepared by combining the ethanolic extracts of *Salvadora persica* and *Andrographis paniculata*. The extracts were mixed in appropriate proportions to ensure optimal antibacterial, anti-biofilm, and anti-inflammatory properties. The final formulation included 2% *Salvadora persica* extract, 2% *Andrographis paniculata* extract, 5% glycerin, 5% sorbitol, 0.1% mint flavor, and distilled water to make up the balance. This combination was carefully designed to harness the therapeutic benefits of both plant extracts while providing a pleasant taste and ensuring the stability of the mouthwash. The inclusion of glycerin and sorbitol not only aids in maintaining the viscosity and texture of the mouthwash but also enhances its moisturizing properties, making it gentle on the oral mucosa. The mint flavor was added to improve the palatability of the formulation, ensuring a refreshing aftertaste(Sushanthi, Doraikannan et al. 2021).

2.3 Bovine serum albumin denaturation assay

The *Salvadora persica* and *Andrographis paniculata*formulation-based mouthwash were tested for their anti-inflammatory activity using two assays: Bovine serum albumin denaturation assay. 0.45 mL of bovine serum albumin was mixed with 0.05 mL of different concentrations (10, 20, 30,40, 50 µg/mL) of *Salvadora persica* and *Andrographis paniculata*formulation-based mouthwash. The pH was adjusted to 6.3. Then it was kept at room temperature for 10 minutes followed by incubation in a water bath at 55°C in a water bath for 30 min. Diclofenac sodium was used as the standard group while dimethyl sulphoxide was used as control. Then, the samples were measured spectrophotometrically at 660nm. The percentage of protein denaturation was determined utilizing the following equation,

% inhibition= $\frac{\text{Absorbance of control- Absorbance of sample}\times 100}{}$

Absorbance of control

2.4 Egg Albumin denaturation assay

To perform the egg albumin denaturation assay, 0.2 mL of fresh egg albumin was mixed with 2.8 mL of 1X phosphate buffer. Different concentrations (10, 20, 30,40, 50 µg/mL) of *Salvadora persica* and *Andrographis paniculata*formulation-based mouthwash were added to the reaction mixture. The pH was adjusted to 6.3. Then it was kept at room temperature for 10 minutes followed by incubation in a water bath at 55°C in a water bath for 30 min. Diclofenac sodium was used as the standard group while dimethyl sulphoxide was used as control. Then, the samples were measured spectrophotometrically at 660nm. The percentage of protein denaturation was determined utilizing the following equation,

% inhibition= Absorbance of control- Absorbance of sample×100 Absorbance of control

2.5 Membrane stabilization assay:

The in vitro membrane stabilization assay is a widely used technique for evaluating the membrane stabilizing properties of natural and synthetic compounds. This assay measures the ability of a compound to stabilize the cell membrane by preventing its disruption and subsequent release of intracellular contents. The materials include Human red blood cells (RBCs), Phosphate-buffered saline (PBS), Tris-HCl buffer (50 mM, pH 7.4), Different concentrations of *Salvadora persica* and *Andrographis paniculata*formulation-based mouthwash (10, 20, 30,40, 50 µg/mL), Centrifuge tube, UV-Vis spectrophotometer(Al-Babtain 2018, Baranikumar, Kumar et al. 2023).

2.6 Preparation of RBC suspension:

Collect fresh human blood in a sterile tube containing anticoagulant. Centrifuge the blood at 3000 RPM for 10 minutes at room temperature to separate the RBCs from other blood components. Remove the supernatant and wash the RBCs three times with PBS. Resuspend the RBCs in the Tris-HCl buffer to obtain a 10% (v/v) RBC suspension

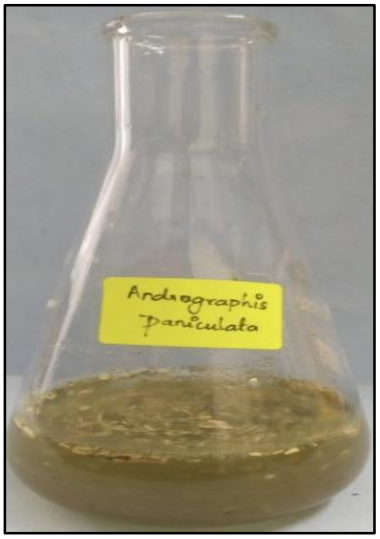
3. Results

3.1 Extraction Results

Figure 1 **Figure 2**

Figure 1: Powdered twigs of *Salvadora persica*

Figure 2: Powdered leaves of *Andrographis paniculata*



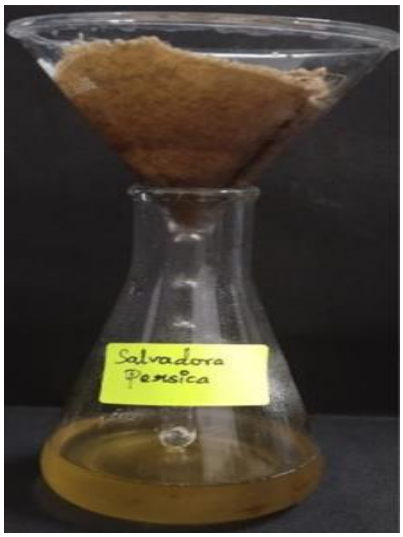
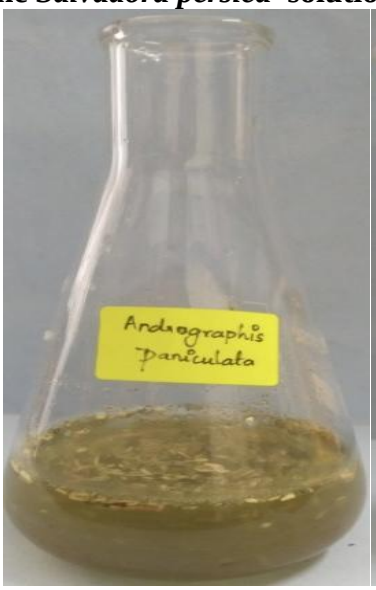


Figure 3 **Figure 4**

Figure 3:*Salvadora persica* extract mixed with distilled water

Figure 4: The *Salvadora persica* solution is filtered using whatman's filter paper



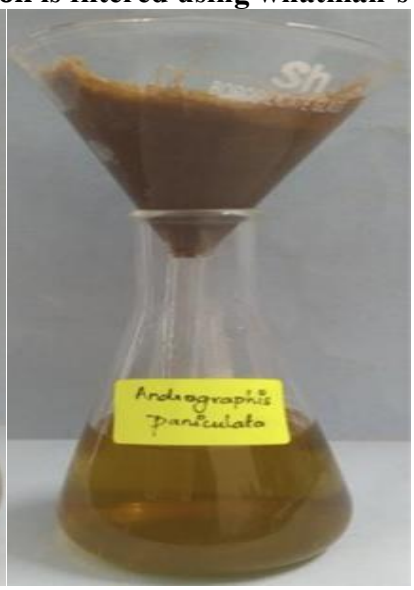


Figure 5 **Figure 6**

Figure 5:*Andrographis paniculata*extract is mixed with distilled water

Figure 6: The *Andrographis paniculata*solution is filtered using whatman's filter paper

3.2 Bovine serum albumin denaturation assay

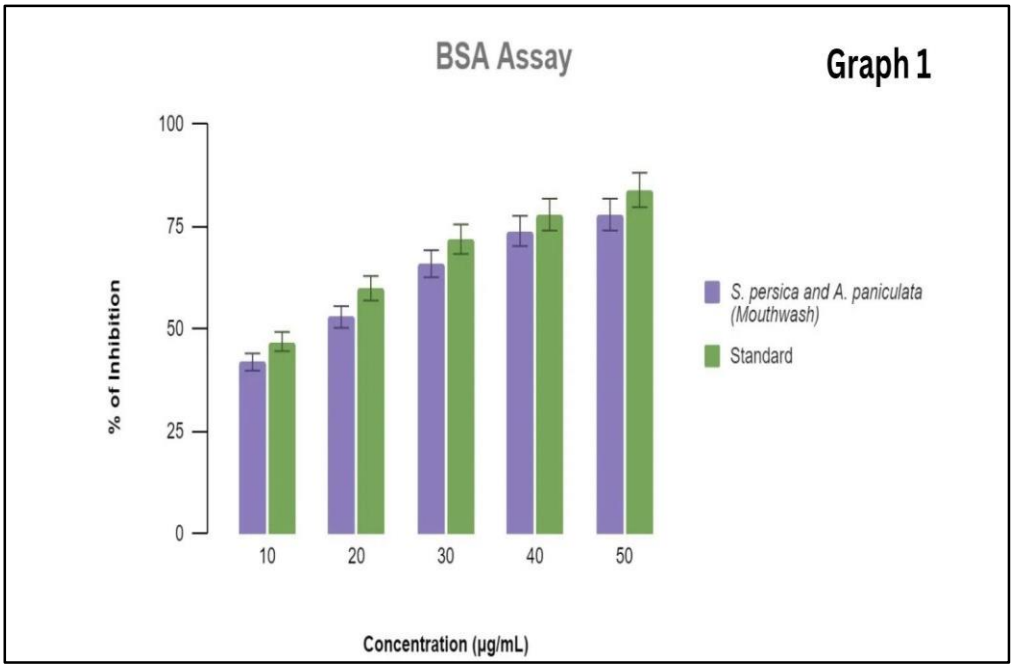


Figure 7:*S.persica*and *A.paniculata* formulated mouthwash and standard mouthwash .

In **Graph 2** [EA Assay], *S. persica* and A. paniculata formulated mouthwash and standard mouthwash.

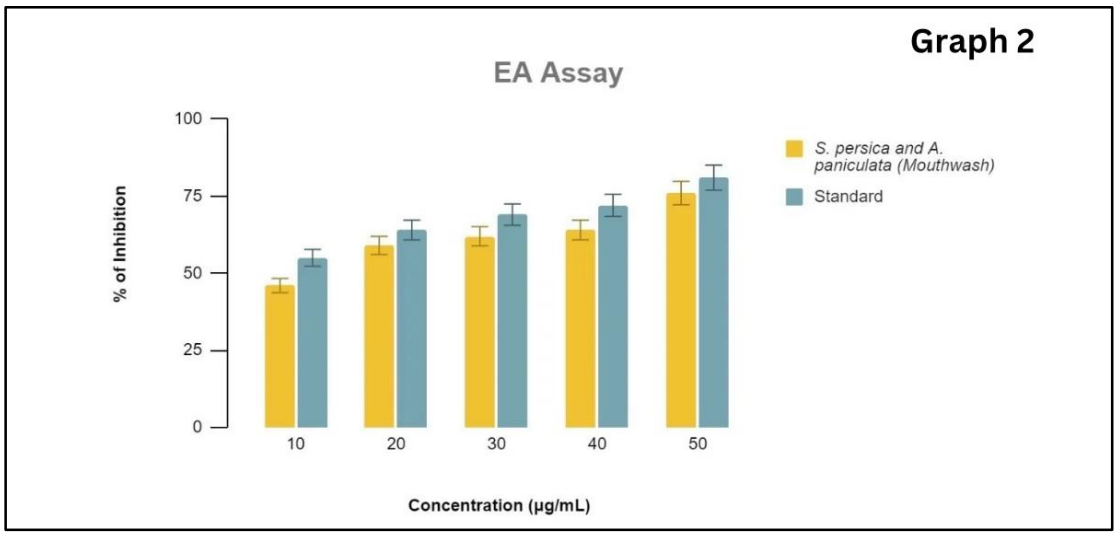


Figure 8: *S. persica* and *A. paniculata* formulated mouthwash and standard mouthwash.

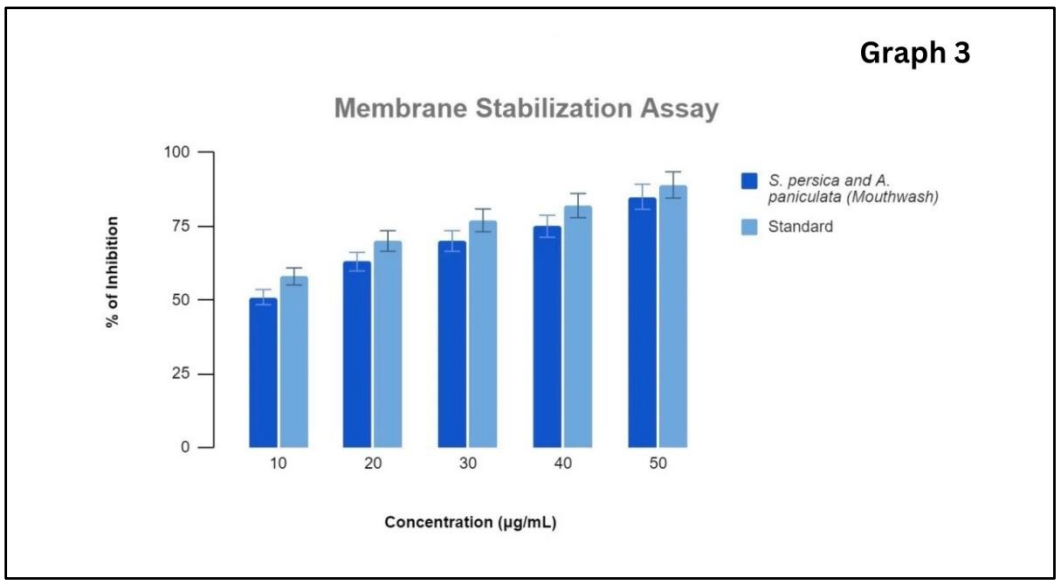


Figure 9:*S.persica* and *A.paniculata* formulated mouthwash is denoted in yellow colour and standard mouthwash is denoted in dark teal colour.

4 Discussion

Our study aimed to evaluate the anti-inflammatory activity of a mouthwash formulated with *Salvadora persica* and *Andrographis paniculata* extracts(Jassoma, Baeesa et al. 2019, Giridharan, Chinnaiah et al. 2024). Graph 1 and Graph 2 demonstrate that the protein denaturation activity of the mouthwash formulated with these extracts is significantly lower compared to the standard mouthwash, indicating its potent anti-inflammatory activity. Graph 3 shows membrane stabilization activity, further supporting the anti-inflammatory potential of the herbal formulation.*Salvadora persica* , also known as the "miracle twig," has been extensively studied in dental science and shown to possess antioxidant and anti-inflammatory properties. These properties contribute to its therapeutic benefits in treating various oral problems, including periodontal diseases and gingival inflammation. Studies have incorporated *Salvadora persica* into mouthwashes, toothpastes, and chewing sticks, highlighting its potential clinical applications in oral health care.The study focused on developing and characterizing a novel oral-herbal mouthwash formula utilizing extracts from *Salvadora persica* (miswak) and *Andrographis paniculata*(Anbarasu, Vinitha et al. 2024, Khalid, Martin et al. 2024). These plants have been traditionally used for oral hygiene due to their antibacterial and anti-biofilm properties. The objective was to create a mouthwash that not only targets oral pathogens effectively but also prevents biofilm formation and reduces inflammation without causing adverse effects.*Salvadora persica* and *Andrographis paniculata* are known for their potent antibacterial properties. The study evaluated the efficacy of the herbal mouthwash against common oral pathogens such as Streptococcus mutans and Porphyromonasgingivalis. Results indicated significant bacteriostatic effects, suggesting that the herbal extracts effectively inhibit bacterial growth crucial for maintaining oral health.Biofilm formation on teeth and oral tissues is a critical factor in dental plaque development and oral disease progression(Ramani, Ravikumar et al. 2022, Raj, Martin et al. 2024). The mouthwash formulation was tested for its ability to inhibit biofilm formation and to disrupt existing biofilms. *Salvadora persica* and *Andrographis paniculata* extracts demonstrated high efficiency in inhibiting biofilm formation, which is crucial for preventing dental plaque accumulation and subsequent gingival inflammation.Protein denaturation is a biochemical process associated with inflammation(Omer, Qarani et al. 2010). The study utilized Bovine Serum Albumin (BSA) and Egg Albumin (EA) as

model proteins to assess the mouthwash's ability to prevent protein denaturation, indicative of its anti-inflammatory potential. Results showed that the herbal extracts in the mouthwash formulation significantly reduced protein denaturation, highlighting their anti-inflammatory properties. This suggests that the mouthwash may help mitigate inflammation in the oral cavity, thereby contributing to overall oral health.An important aspect of the study was to evaluate the safety profile of the herbal mouthwash. Traditional oral hygiene products sometimes pose risks such as mucosal irritation or allergic reactions. However, the formulated mouthwash showed little to no unintended side effects, indicating its safety for oral use. This is crucial for promoting patient compliance and long-term use of the product.The findings of this study have significant clinical relevance in the field of oral health care. The herbal mouthwash formulation offers a natural alternative to conventional chemical-based mouthwashes. Its dual action against bacteria and biofilms, coupled with anti-inflammatory properties, positions it as a promising therapeutic option for maintaining oral hygiene and preventing dental diseases.Future research could focus on conducting clinical trials to validate the efficacy and safety of the herbal mouthwash in human subjects. Long-term studies could also assess its impact on oral microbiota balance and its potential role in preventing periodontal diseases(Prathap , Abdullah, Niazy et al. 2016). Furthermore, investigating the mechanism of action of *Salvadora persica* and *Andrographis paniculata* extracts at the molecular level could provide deeper insights into their therapeutic benefits.

5 Conclusion

In conclusion, the study successfully developed and characterized an oral-herbal mouthwash formulation containing *Salvadora persica* and *Andrographis paniculata* extracts. The formulated mouthwash demonstrated significant antibacterial activity against oral pathogens, effective inhibition of biofilm formation, and notable anti-inflammatory properties as evidenced by reduced protein denaturation. Moreover, it exhibited a favorable safety profile with minimal unintended side effects. This research underscores the potential of herbal extracts in oral care, offering a natural and effective approach to promoting oral health and preventing oral diseases.

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