JBP
Journal of Biochemicals and Phytomedicine
eISSN: 2958-8561

Development of A Daily Oral Hygiene Product with Zinc Sulfate

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ARTICLE INFO

Article Type:
Research

Article History:
Received: 29 Jan 2025
Revised: 12 Mar 2025
Accepted: 3 Jun 2025
Available online: 23 Sep 2025

Keywords:
Anti-infective agents,
Zinc sulfate,
Clove oil,
Oral health,
Dental plaque,
Microbiota

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ABSTRACT

Introduction: Proper oral care is essential for overall health. The effectiveness of daily hygiene depends on its thoroughness and personalization. Without regular cleaning, the oral cavity accumulates pathogenic microbes, damaging soft tissues and hard tooth structures, compromising bodily integrity. This study aimed to evaluate oral hygiene products and develop an effective daily-use preventive rinse.

Methods: We reviewed studies on dental diseases and hygiene products via PubMed, Scopus, and Web of Science. The rinse was developed considering ingredient properties and regulatory guidelines. Microbiological safety, organoleptic, and physicochemical properties were tested.

Results: Mouthwashes enhance oral hygiene by mechanically cleaning teeth, gums, and interdental spaces, removing debris and pathogens. Regular use prevents disease and improves tissue blood flow. Based on existing evidence, we formulated a rinse containing zinc sulfate (inhibits plaque and calculus) and clove oil (antimicrobial and antioxidant). A scalable production process was designed. Quality was assured through organoleptic, physicochemical, and microbiological tests.

Conclusion: The developed antimicrobial and anti-inflammatory rinse is suitable for daily use, improving interdental cleaning.

Please cite this paper as:
Hrynovets IS, Storozhynska MV, Lysiuk RM. Development of a daily oral hygiene product with zinc sulfate. Journal of Biochemicals and Phytomedicine. 2025; 4(2): 26-33. doi: 10.34172/jbp.2025.14.

Introduction

Daily oral hygiene comprises preventive measures essential for maintaining oral health and preventing dental pathology. Inadequate oral hygiene practices are frequently associated with improper selection of hygiene products (Mathur et al., 2019; Rajasekaran et al., 2024). The individual spatial arrangement of dentition allows food debris to accumulate interdentally, while plaque forms in areas inaccessible to toothbrushes. These retained residues undergo oxidation when moistened by oral fluid, altering oral pH and creating a favorable environment for pathogenic microorganism proliferation (Courtois, 2021). Such microbial shifts can adversely affect systemic health, serving as triggering factors for local diseases, modulating immune responses, and impairing dietary nitrate metabolism. The activation of local oral factors, including altered salivation, compromised immune response, and rapid expansion of pathogenic microflora, can initiate and propagate disease processes (Nijakowski et al., 2021). This may lead to conditions such as aphthae, stomatitis, or caries, accompanied by reduced lysozyme concentrations in saliva. For instance, dental caries involves significant biochemical alterations in saliva's mineral composition, causing trophic disturbances at cellular and tissue levels that, combined with microbial factors, contribute to severe dental pathologies (da Silveiraa et al., 2024).

Poor oral hygiene is directly associated with several common oral conditions:

Dental caries, characterized by enamel demineralization, typically results from excessive sugar consumption and carbohydrate metabolism disorders, leading to localized acidification, pathogenic microflora expansion, and disruption of mineralization homeostasis (Tanner et al., 2018).

Gingivitis, a reversible inflammatory gum condition often presenting with bleeding during brushing, originates from biofilm accumulation at the gingival margin and resolves with effective plaque removal (Lamont et al., 2018).

Stomatitis, manifesting as mucosal inflammation with erythema, pain, ulceration, and swelling, can be initiated by infectious agents or poor oral hygiene that promotes pathogenic microorganism growth (Lamont et al., 2018).

Periodontitis, a chronic inflammatory disease destroying periodontal supporting tissues, demonstrates complex etiology involving dysbiotic microbial communities, host genetic predisposition, and environmental factors that collectively modulate immune responses (Hajishengallis, 2015). While dysbiosis alone may not precipitate periodontitis, it can initiate disease progression in conjunction with other risk factors. Figure 1 illustrates the potential developmental pathways of hard and soft tissue diseases in the oral cavity.

Zinc demonstrates multifunctional roles in human health, particularly noted for its antibacterial and antiviral properties. The recent COVID-19 pandemic emphasized oral hygiene's significance in viral transmission dynamics. The oral cavity serves as an entry portal for opportunistic pathogens like SARS-CoV-2, detectable in saliva of infected individuals. Viral loads peak during initial infection and decline with clinical improvement, indicating correlation between salivary viral secretion and disease manifestation (Bajaj et al., 2020). Viral infections including COVID-19 can induce hyposalivation, further compromising host defenses through reduced enzymatic activity and lysozyme concentration. SARS-CoV-2 utilizes angiotensin-converting enzyme (ACE) receptors expressed in oral tissues via spike protein binding (Herrera et al., 2020), facilitating cellular entry, replication, and cytolysis, ultimately causing oral symptoms (Sakaguchi et al., 2020). Periodontal pockets may serve as reservoirs for SARS-CoV-2, enabling viral replication, oral cavity dissemination, and systemic spread via periodontal vasculature (Badran et al., 2020). Consequently, effective oral hygiene practices are crucial for reducing opportunistic pathogen loads and potentially mitigating disease transmission rates.

The critical importance of oral hygiene demands careful selection of appropriate products and instruments. Contemporary markets offer numerous hygiene aids—toothbrushes, dental floss, irrigators, and stimulators—alongside various formulations including toothpastes, gels, powders, chewing gums, and rinses. Mouthwashes represent supplementary liquid hygiene products that assist in reducing pathogenic microflora, pH adjustment, cleansing inaccessible areas, and breath freshening. Scientific literature conventionally classifies mouthrinses into preventive and therapeutic categories based on their functional properties, as detailed in Figure 2.

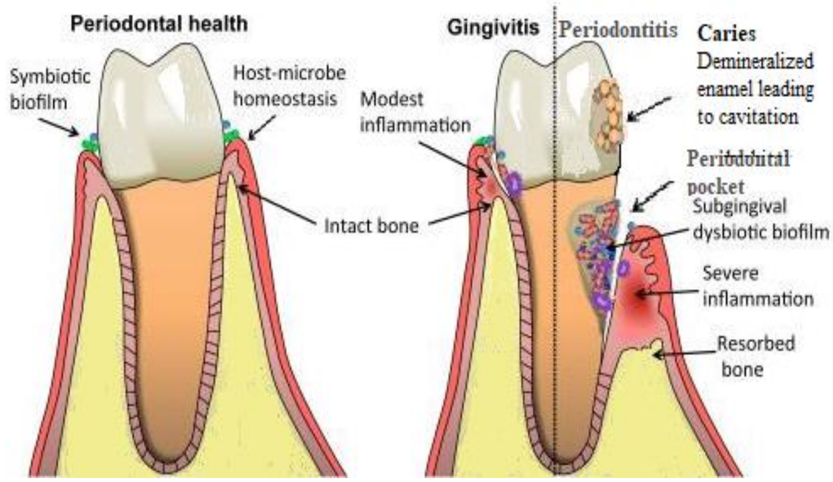

Figure 1. Potential progression pathways of hard and soft tissue diseases in the oral cavity (authors' own elaboration)

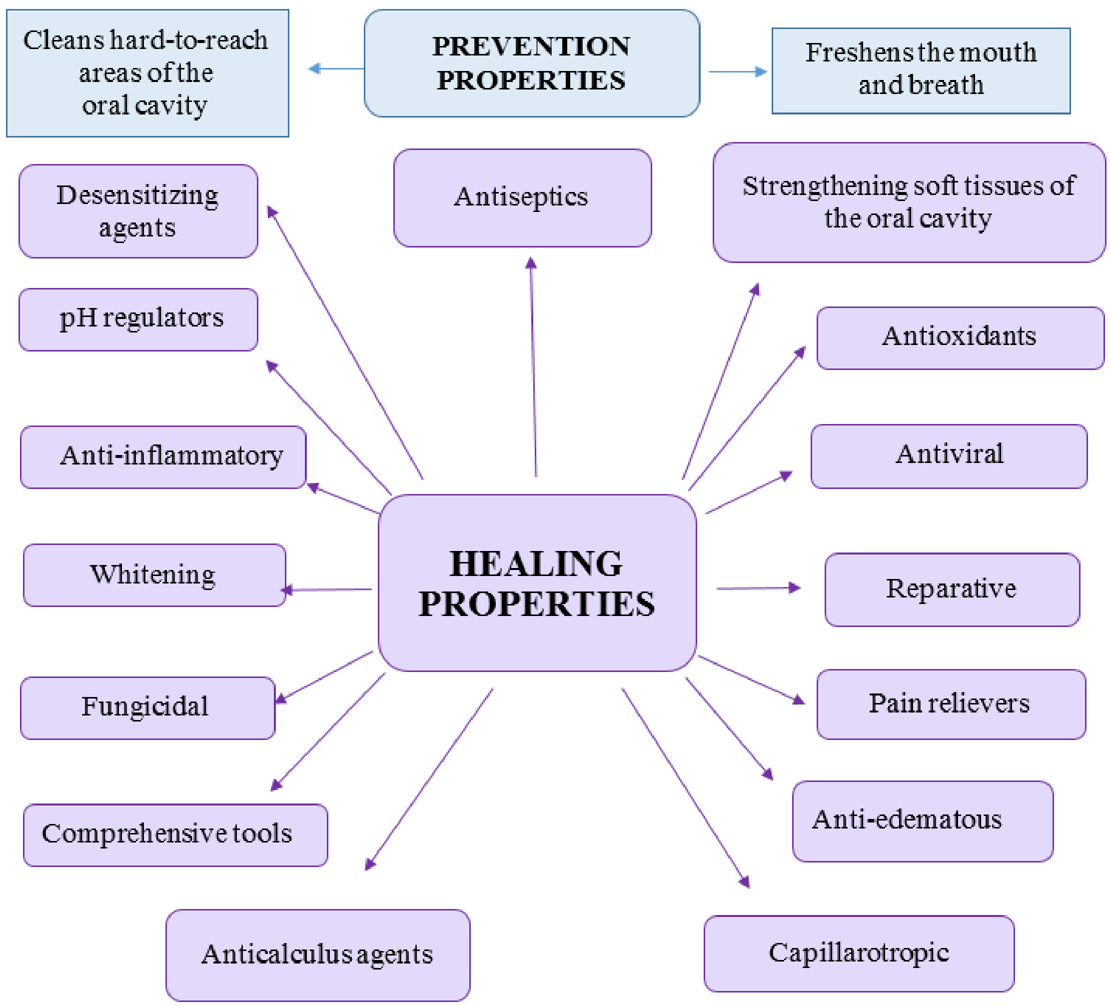

Figure 2. Classification of mouthwash properties based on preventive and therapeutic functions (authors' own elaboration)

Despite the wide availability of mouthwash products, this study developed a novel formulation incorporating zinc sulfate and clove essential oil as active components, previously demonstrated effective against pathogenic microflora (Hrynovets et al., 2024a). The primary research objective involved developing this mouthwash and comprehensively evaluating its physicochemical, organoleptic, and microbiological properties to establish an effective, safe, and organoleptically acceptable adjunct to daily oral hygiene, particularly valuable in situations where mechanical cleaning proves insufficient.

Methods

A review and analysis of scientific literature from databases including PubMed, Scopus, and Web of Science were conducted to inform the pharmaceutical development of an oral hygiene formulation. As no standardized protocol existed for the combination of zinc sulfate and clove oil, experimental procedures were designed de novo. Two independent series of the mouth rinse, containing zinc sulfate and clove oil as principal active components, were produced under laboratory conditions using a standardized sequence of technological steps to ensure reproducibility and solution homogeneity.

During development, zinc sulfate concentrations from 0.05% to 0.2% (0.05%, 0.1%, 0.15%, 0.2%) were tested. The upper limit was determined based on safety recommendations from the FDA, EMA, INCI, and the EU Cosmetic Ingredient Database. Microbiological purity and safety studies indicated that a 0.1% zinc sulfate concentration was optimal. The concentration of clove essential oil was set at 0.09 ± 0.01 g, equivalent to two drops from a standard dropper, complying with the State Pharmacopoeia of Ukraine.

The extemporaneous manufacturing technology followed a defined step-by-step procedure. Preparatory work involved organoleptic checks of ingredients, weighing, and equipment preparation. The final formulation per 100 mL was: Zinc sulfate (0.1 g), Sodium carboxymethyl cellulose (1.0 g), Glycerin (6.0 g), Propylene glycol (3.0 g), Saccharin (0.1 g), Clove essential oil (0.09 g), Menthol (0.05 g), and Purified water (q.s. to 100.0 mL).

The technological process consisted of (Figure 3):

Stage 1: Preparation of sodium carboxymethyl cellulose solution (30 ± 5 °C, 45 min)

Stage 2: Preparation of zinc sulfate solution

Stage 3: Preparation of saccharin solution (100 °C, 0.5 min)

Stage 4: Mixing clove oil with glycerin and propylene glycol

Stage 5: Combining all solutions and adding menthol (30 °C)

Final stages: Filling into sanitized containers, labeling, and packaging

Organoleptic characteristics and pH were evaluated in triplicate. The mouthwash underwent organoleptic, physicochemical, and microbiological testing following Ukrainian regulations (DSTU 4186:2003 and State Sanitary Rules No. 27).

Results

The developed mouthwash was standardized, and key quality and safety criteria were established for its use as a daily preventive product. Two rinse series were compared: one freshly prepared and one stored for six months at 20 ± 5 °C.

Both liquids were homogeneous, transparent suspensions with minimal sediment that dissipated upon shaking. The solution color was opaque transparent with a slight white tint, characteristic of its components. The odor was indicative of clove oil and menthol, and the taste was pleasant and slightly sweet. The glycerin number was 6.0 ± 2, and the pH was 7.0 ± 1.

Microbiological purity was assessed against National Standards of Ukraine. Table 1 outlines microbiological safety parameters for oral mucosa-contact products, stratified by user sensitivity, while Table 2 details organoleptic and physicochemical characteristics confirming adherence to quality standards.

As shown in Figure 3, a technological scheme for industrial production was developed, with critical phases highlighted. These results confirm the product meets the required safety and quality benchmarks for an effective oral hygiene product.

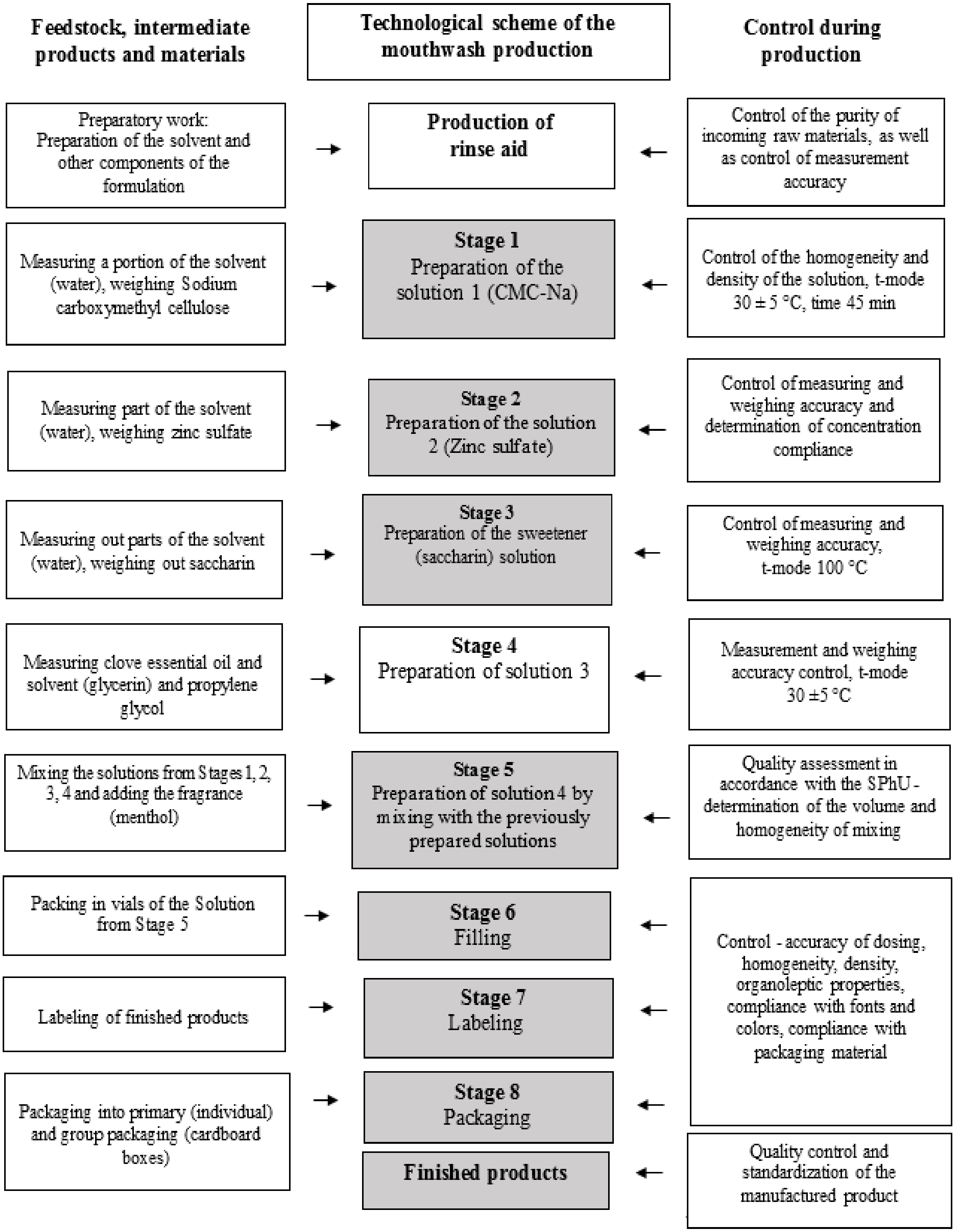

Figure 3. Flowchart of the manufacturing process for zinc sulfate-clove oil mouthwash.

Table 1. Microbiological purity specifications for oral mucosa-contact hygiene products according to Ukrainian regulatory standards.

Name of the indicator	Unit of measure	Norm
Number of mesophilic aerobic and facultative anaerobic microorganisms, not more than	CFU/cm3	1000
Bacteria of the family Enterobactereaceae	1 cm3	Absent
Bacteria Staphylococcus aureus	1 cm3	Absent
Bacteria Pseudomonas aeruginosa	1 cm3	Absent
Number of yeasts and molds, not more than	CFU/cm3	100

Sources (Badran et al., 2020; The National Standard of Ukraine DSTU 4186:2003).

Table 2. Organoleptic and physicochemical characteristics of the developed mouthwash formulation.

Name of the indicator	Characteristics and standards
Appearance	Homogeneous single-phase or multiphase liquid without impurities
Colour	The presence of slight turbidity or sediment is permissible
Odour	Must match the odour of the product of a certain name
Taste	Must match the taste of the product of a certain name
Hydrogen index (pH)	Pleasant

Source (The National Standard of Ukraine DSTU 4186:2003**).**

Discussion

Zinc sulfate has several effects on oral bacteria and inhibits numerous enzymes in bacterial cells. It can increase the proton permeability of bacterial cells and inhibit the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase (Badran et al., 2020). Zinc is also a component of several antiviral enzymes, such as proteases and polymerases. It is a cofactor for antioxidant enzymes such as superoxide dismutase and also induces the synthesis of metallothionein, which is a cysteine (sulfhydryl-rich) protein that protects cells from free radicals, supporting cellular immunity (Ashok et al., 2020). It also reduces oxidative stress caused by reactive oxygen species generated by mitochondrial dysfunction or during viral infections by regulating the release of metallothionein (Alpert, 2017). Zinc sulfate is included in oral care products to inhibit the growth of cariogenic bacteria, control plaque formation, and reduce bad breath (Hrynovets et al., 2024a).

The substance has antimicrobial and antioxidant effects. The antibacterial mechanism is associated with -OH groups located in meta- and ortho-positions. These functional groups can interact with the cytoplasmic membrane of microbial cells. The active principles penetrate through the cell membrane due to its lipophilic properties. Interacting with polysaccharides, fatty acids and phospholipids, cause loss of cell membrane integrity, leakage of cellular contents and inhibition of the activity of the proton pump, which lead to cell death. It can inhibit gram-negative bacteria (Escherichia coli, Klebsiella pneumoniae, Salmonella spp, Agrobacterium spp and Pseudomonas aeruginosa) and gram-positive bacteria (Staphylococcus aureus, Listeria monocytogenes, Streptococcus mutans and S. sanguinis) and protect cells from free radical oxidation (Othman et al., 2019; Ginting et al., 2021).

The hydroxyl group, present in the aromatic ring of eugenol molecule, is responsible for antioxidant activity, and phenolic compounds transfer electrons or hydrogen atoms and neutralize them to free radicals, which leads to blocking the oxidative process and death of the pathogenic cell. Due to its structure, eugenol is active against the SARS-CoV-2 virus because it penetrates the lipid bilayer of the virus envelope, inhibits the enzymatic activity of the virus, which leads to the viral death (Haro-González et al., 2021).

Based on the study of available scientific and informational materials, it can be concluded that the concentration of zinc sulfate in the composition of hygiene products within 0.1% is optimal; the same concentration is used in registered hygiene products available on the market currently, such as Listerine Total Care Zinc, Meridol Halitosis Mouthwash and Parodontax Extra, as well as in liquid forms manufactured not only industrially but also extemporaneously and practically applied in dental practice for aphthae, stomatitis, gingivitis, periodontal disease and other diseases.

Microbiological purity is a key indicator of the safety of oral care products. Failure to comply with it can not only negate the hygienic effect, but also cause infectious complications.

Therefore, determining the microbiological purity of oral care products (toothpastes, rinses, sprays, etc.) is critical because these products :

- contaminated with microorganisms can cause inflammation, stomatitis, glossitis, periodontitis in contact with the oral mucosa, which is very sensitive and easily infected;

- can become a source of pathogenic microorganisms, since if the product is contaminated with pathogenic or opportunistic bacteria (e.g., Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli), it poses a real danger, especially for people with reduced immunity;
- have a prolonged contact with the consumer considering that the products are used daily and for a long time, so even low microbial contamination can gradually cause an imbalance in the oral microflora or allergic reactions.

The main limitations of the study comprise the used only affordable full-text publications available in English and application of the related regulatory and technical documentation requirements, acting in Ukraine, during the formulation development.

The future trends of the research are aimed at the preclinical and further clinical studies of the developed formulation.

The hygienic mouthwash in liquid dispersion form plays a key role not only in the general complex of daily hygiene procedures, but is also indispensable in clinical cases in which the use of a toothbrush and toothpaste is impossible. For example, in occur a jaw fracture with the corresponding splint/wire construction; therapeutic periods after tooth extraction, bone resection, and various implants; during periodontal treatment, which involves deep cleaning of the pocket at the neck of the tooth and during curettage; in orthodontics, when braces, plates, veneers and other structures are used; for cancer patients during chemotherapy or radiation therapy; and also for a group of patients with limited physical activity, such as those who is having emergency conditions or neurological disorders, when a person cannot perform hygiene procedures on their own.

The therapeutic potential of this formulation derives from the complementary mechanisms of its constituent agents. Zinc sulfate exhibits immunomodulatory capabilities through the enhancement of natural killer cell responsiveness, augmentation of phagocytic efficiency, and regulation of immune cell populations. Concurrently, clove essential oil delivers antimicrobial and antioxidant actions that effectively suppress pathogenic microbial proliferation. This combination yields a comprehensive oral hygiene product capable of addressing both mechanical debridement needs through interdental cleaning and biological protection through immunomodulation and microbial control.

Conclusion

This research establishes the successful development of a novel mouthwash formulation utilizing zinc sulfate and clove essential oil as key active ingredients. Through systematic evaluation, the extemporaneously prepared solution demonstrated favorable organoleptic characteristics, appropriate physicochemical parameters, and compliance with microbiological safety standards. A defined manufacturing protocol, structured around eight critical stages, was established to ensure consistent production quality.

Declarations

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

Not applicable.

Consent for publications

All authors have read and approved the manuscript for publication.

Funding/support

This work received no funding.

Authors' contributions

IH and MS initiated the research concept and developed the overall framework. IH, MS, and RL conducted all experiments and prepared the initial draft of the manuscript, while IH and MS performed the literature survey. MS and RL carried out the formal analysis, data curation, and visualization. IH was responsible for funding acquisition, project administration, resources provision, and overall supervision of the research. MS also contributed to software implementation. Validation of the results was performed by IH and MS. Finally, RL reviewed and edited the manuscript. The final version of the manuscript was read and approved for publication by IH, MS, and RL.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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