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A comparative analysis of antimicrobial agents in artificial saliva: Scoping review

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Abstract

Background: Artificial saliva should be formulated to mimic the physical properties and composition of the natural saliva. In addition to its moisturizing effects, artificial saliva should also include buffering and antimicrobial properties to adequately substitute the physiological functions of natural saliva. Most artificial saliva products focus on lubrication and moisture retention, with limited exploration of antimicrobial components. The optimal antimicrobial agents in artificial saliva have yet to be determined.

Purpose: To identify antimicrobial agents used in artificial saliva.

Methods: Article searches were conducted using the PRISMA flow chart in October 2024 using three databases: Scopus, Pubmed, and ScienceDirect. Selected articles were then evaluated for risk of bias using the JBI critical appraisal checklist.

Results: Six quasi-experimental studies met the inclusion criteria and included in this review.

Conclusion: Antimicrobial agents used in artificial saliva are xylitol, sorbitol, lysozyme, lactoferrin, lactoperoxidase with potassium thiocyanate, hinokitiol, protamine, whey protein, dried egg yolk, system of enzymes, fluoride ions, hydroxyethylcellulose, and core-shell magnetic nanoparticles. Various other active ingredients may also serve as antimicrobial agents.

Keywords: Artificial saliva; Antimicrobial; Antifungal; Antibacterial; Xerostomia

1. Introduction

Saliva is an exocrine fluid produced by the salivary glands containing 99% water and 1% protein and electrolytes. The salivary glands synergistically secrete saliva, contributing to various physiological processes and oral functions. Saliva lubricates the mouth, helps in swallowing and chewing, supports speech, enhances taste perception, and provides antimicrobial defense [1,2]. Normally, the average daily flow of the major and minor salivary gland secretion in healthy adults is approximately 1000–1500 mL/day. Various causes such as aging, side effects of radiation therapy to the head and neck, medication intake, and sufferers of Sjögren's syndrome can lead to hyposalivation and/or xerostomia. If the salivary secretion rates are below designated thresholds, the patient is diagnosed with hyposalivation, meanwhile xerostomia can be interpreted as a subjective dry mouth sensation [3,4]. This subjective sensation is not only reflected in the reduced amount of saliva but also the composition and quality of the saliva [5]. The prevalence of xerostomia ranges from 0.9% to 64.8% worldwide [6,7]. Xerostomia can manifest in various ways, from mild oral irritation to severe oral conditions that may impact a patient's overall health, dietary intake, and quality of life [8,9].

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Currently, the management of xerostomia involves pharmacological and/or palliative approaches. Pharmacotherapy commonly includes the use of sialogogues, such as pilocarpine or cevimeline, to stimulate saliva secretion by the salivary glands [10,11]. Palliative treatment focuses on enhancing oral lubrication through increased water intake, chewing gum to promote salivary stimulation, and the use of oral moisturizer such as artificial saliva or mouth rinses to alleviate oral dryness [10,12].

Artificial saliva is a moisturizing agent for the oral cavity, available in liquid, spray, or mouthwash forms, designed to mimic the physical properties and composition of the natural saliva [8]. Beyond its moisturizing function, artificial saliva should also provide buffering and antimicrobial properties to effectively replace the physiological roles of natural saliva. Given the common prevalence of dental caries and candidiasis in patients with xerostomia, the therapeutic use of artificial saliva should include sufficient antibacterial and antifungal agents [13].

Although various antimicrobial and antifungal components are used in various studies and products, antimicrobial agents in artificial saliva that work optimally to maintain the balance of the oral microbiome have not been widely studied. Most research and commercially available products for artificial saliva have focused on physical properties like moisture retention and lubrication. However, there has been little investigation into the potential of antimicrobial components in artificial saliva to reduce the risk of opportunistic infections in the oral cavity, such as oral candidiasis or dental caries, especially in patients with xerostomia. Therefore, this study aims to identify antimicrobial agents used in artificial saliva. The findings are anticipated to contribute to the further research focused on optimizing the efficacy and therapeutic properties of artificial saliva, developing more effective solutions for individuals suffering from xerostomia, thereby enhancing their oral health and overall quality of life.

2. Material and methods

Once the topic was determined, a searching process for relevant articles was carried out. Three databases: Scopus, Pubmed, and ScienceDirect, were employed to gather literature for this scoping review. The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 template guided the selection of articles and data extraction from articles that met the review's inclusion criteria. The article search utilized the PEO framework, which includes (1) Population/Problem: The population or issue relevant to the scoping review topic that will be examined; (2) Exposure: The condition or exposure experienced by the patients/population; and (3) Outcome: The findings from previous studies that correspond with the exposure.

In this review, the PEO framework identified strains of bacteria or fungi as the Population, artificial saliva with antimicrobial agents for xerostomia therapy as the Exposure, and antimicrobial effects as the Outcome. The literature search process was conducted in October 2024 for preliminary data. Boolean operators were applied to refine keywords (AND, OR) for the article searches, enabling a more targeted or broad search approach for easier article retrieval. Table 1 below outlined the keyword combinations used with boolean operators.

Table 1 Keywords used for A Comparative Analysis of Antimicrobial Agents in Artificial Saliva: Scoping Review

Artificial Saliva		**Antimicrobial**
Artificial saliva		Antimicrobial
OR		OR
Saliva substitutes	AND	Antifungal
OR		OR
Oral moisturizer		Antibacterial

Following the article search, relevant studies that met the criteria were identified. All studies retrieved from the database based on the specified search criteria were compiled, and duplicates were eliminated. The remaining studies were filtered by reviewing their titles and abstracts, which led to the exclusion of those that did not fit the inclusion criteria. In the final step, the full texts of the remaining studies were examined, and any that did not align with the inclusion criteria were excluded. Consequently, the final articles were selected for inclusion in this review.

Based on the topic of this scoping review, the data collected must include information on the composition of artificial saliva samples and antimicrobial components (antifungal or antibacterial, or both) in artificial saliva. The inclusion criteria used in this scoping review were (1) Includes the composition of artificial saliva samples; (2) Includes antimicrobial components (antifungal or antibacterial, or both) in artificial saliva; (3) Open access journal article; (4) Research article in English. While the exclusion criteria in this scoping review included (1) Literature with narrative review, systematic review, and meta-analysis research types; (2) Full-text articles that cannot be accessed. (3) Research using languages other than English.

3. Results and discussion

Through the data search across three databases: Scopus, PubMed, and ScienceDirect, a total of 27,333 articles were obtained. Screening for duplicates was conducted, leaving 13,459 articles remaining. Subsequently, screening based on titles resulted in 78 articles then followed by abstracts screening, resulting in 21 articles. From these 21 articles, full-text screening was conducted against the inclusion and exclusion criteria, resulting in inclusion of 6 articles (Table 2) and the exclusion of 15 others. This process is illustrated in the following PRISMA diagram (Fig-1).

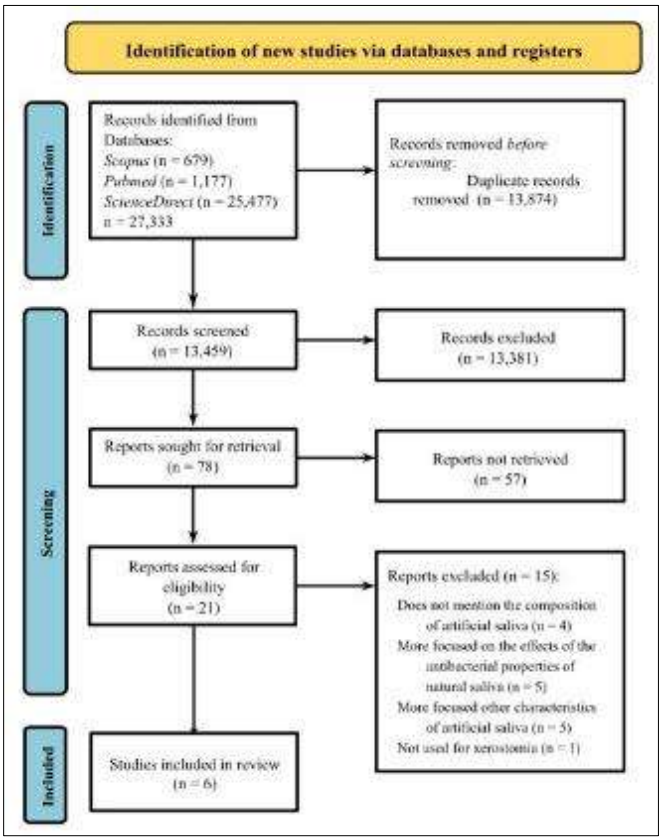

Figure 1 PRISMA Diagram flow

Table 2 Included studies

No	Reference	Study Design	Population	Antimicrobial Assay
1	(Murakami et al., 2018)	Quasi experimental	*Candida albicans*	MIC
2	(Kang et al., 2017)	Quasi experimental	*Candida albicans* (ATCC 10231) *Actinomyces viscosus* (ATCC 15987) *Staphylococcus aureus* (ATCC 6538P)	Disk diffusion method
3	(Łysik et al., 2020)	Quasi experimental	*Candida albicans* *Candida glabrata*	CV-staining MTT

			Candida tropicalis *Streptococcus mutans*	
4	(Murakami et al., 2020)	Quasi experimental	*Candida albicans* (JCM1537) *Candida glabrata* (JCM3699)	MIC
5	(Niemirowicz-Laskowska et al., 2020)	Quasi experimental	*Streptococcus mutans*	CV-staining MTT
6	(Altin et al., 2021)	Quasi experimental	*Streptococcus mutans*	MIC

The 6 included studies are quasi experimental thus the risk of bias was assessed using JBI critical appraisal for quasi experimental studies (Table 3). The assessment resulted in 8 out of 9 checklists fulfilled. Therefore, the studies are considered having low risk of bias and eligible for inclusion [14].

Table 3 Risk of bias assessment for the included studies

No	**Reference**	**Questions**									**Conlusion**
		1	2	3	4	5	6	7	8	9	
1	(Murakami et al., 2018)	√	√	√	√	X	√	√	√	√	Included
2	(Kang et al., 2017)	√	√	√	√	X	√	√	√	√	Included
3	(Łysik et al., 2020)	√	√	√	√	X	√	√	√	√	Included
4	(Murakami et al., 2020)	√	√	√	√	X	√	√	√	√	Included
5	(Niemirowicz-Laskowska et al., 2020)	√	√	√	√	X	√	√	√	√	Included
6	(Altin et al., 2021)	√	√	√	√	X	√	√	√	√	Included

Description: √ = in accordance; X = not in accordance

Questions related to risk of bias in the table:

1. Is it clear in the study what is the "cause" and what is the "effect" (ie, there is no confusion about which variable comes first)?
2. Was there a control group?
3. Were participants included in any comparisons similar?
4. Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?
5. Were there multiple measurements of the outcome, both pre and post the intervention/exposure?
6. Were the outcomes of participants included in any comparisons measured in the same way?
7. Were outcomes measured in a reliable way?
8. Was follow-up complete and, if not, were differences between groups in terms of their follow-up adequately described and analyzed?
9. Was appropriate statistical analysis used?

From the 6 included studies, the dosage form, main ingredients, antimicrobial agents, and antimicrobial effect of artificial saliva used was summarized in Table 4. The components that make up artificial saliva in the inclusion study are varied in the forms solution and gel. The resulting antimicrobial effect in artificial is an antibacterial or antifungal effect, or both.

Table 4 Dosage form, main ingredients, antimicrobial agents, and antimicrobial effect of artificial saliva

No	Reference	Dosage form	Main ingredients	Antimicrobial agents	Antimicrobial effect
1	(Murakami et al., 2018)	Solution	Water, protamine resolution peptide, glycerin, sucralose, xylitol, cetylpyridinium, whey protein, lactoferrin	Hinokitiol Protamine Whey Protein	Antifungal
		Gel	Water, hyaluronate sodium, glycerin, xylitol, hinokitiol		
2	(Kang et al., 2017)	Solution & gel	Potassium chloride Magnesium chloride Sodium chloride Potassium thiocyanate Lysozyme Calcium chloride Glucose oxidase Lactoperoxidase	Potassium thiocyanate Lactoperoxidase Lysozyme	Antifungal Antibacterial
3	(Łysik et al., 2020)	Solution	Mucin Xylitol Xanthan gum	Xylitol	Antifungal Antibacterial
4	(Murakami et al., 2020)	Solution	Water, Sorbitol, Xylitol, PG, Whey protein, Lactoferrin	Hinokitiol Whey protein Lactoferrin Dried egg yolk	Antifungal
		Gel	Water, Hyaluronate sodium, Glycerin, Xylitol, Hinokitiol		
			Water, Glycerin, Sorbitol, Dried egg yolk, Xanthan gum		
			Water, Xylitol, Sorbitol, Maltitol, Glycerin, Whey protein		
			Water, Xylitol, Glycerin, Maltitol, Sorbitol, Dried egg yolk		
5	(Niemirowicz-Laskowska et al., 2020)	Solution	Xylitol, hydroxyethylcellulose, poloxamer, system of enzymes	Xylitol Sorbitol Fluoride ions System of enzymes Hydroxyethylcellulose Core-shell magnetic nanoparticles (gold-coated and aminosilane-coated nanoparticles NPs	Antifungal Antibacterial
			Xylitol, cellulose gum, glycerine		
			Sorbitol, Yerba Santa extract		
6	(Altin et al., 2021)	Solution	Sodium chloride Potassium chloride Magnesium chloride KOH Calcium chloride	Lysozyme Lactoferrin	Antibacterial

			Methylcellulose Phosphoric acid Lactoferrin Lysozyme		

Artificial saliva aims to replicate the functions of natural human saliva as closely as possible, particularly in moisturizing and lubrication within the oral cavity. Therefore, artificial saliva typically consists of a base formulated with polymers or glycerol, including polysaccharides, mucins, or cellulose derivatives, which serve as moisturizing and lubricating agents [15]. Some artificial saliva formulations also include gelling agents to create gel-like preparations. Furthermore, an important function that also must be mimicked from natural saliva is its antimicrobial activity.

Based on the included studies, the antimicrobial agents used in artificial saliva reviewed are primarily xylitol, lysozyme, lactoferrin, and hinokitiol. Each of these components provides certain antifungal and antibacterial actions, contributing to the antimicrobial efficacy of artificial saliva. In xerostomia cases where natural antimicrobial components are reduced, these agents are important to prevent infection. Additionally, combinations of certain components may create synergistic effects to enhance oral cavity protection against microbes.

Xylitol as antimicrobial agents used in artificial saliva was mentioned in the studies of Łysik et al. (2021) and Niemirowicz-Laskowska et al. (2020). Łysik et al. (2021) proposed a xylitol-enriched mucin-based saliva substitute and concluded that xylitol at a concentration of 1–5% in artificial saliva can significantly improve the antimicrobial properties. Artificial saliva with xylitol exhibits the ability to prevent adhesion, proliferation, and biofilm formation of *Candida* and *S. mutans*. The reduction of *Streptococci* occurs by disrupting energy production pathways and causing internal cell damage. Transporting sugars into bacterial cells requires energy and is essential for metabolism; while glucose undergoes fermentation to generate energy, xylitol is transformed into xylitol-5-phosphate, which does not produce energy. Instead, this conversion leads to the formation of vacuoles and the degradation of the cell membrane, ultimately resulting in cell death. Dephosphorylated xylitol is subsequently removed from the cell through an energy-consuming process. Additionally, xylitol inhibits fungal growth because Candida cells cannot metabolize it, causing accumulation in the cytoplasm and increasing osmotic pressure within the cell [16,17].

Niemirowicz-Laskowska et al. (2020) enhanced commercial artificial saliva by incorporating active ingredients. Their initial assessment of the antimicrobial properties of these formulations revealed that some components, such as Yerba Santa extract and hydroxyethylcellulose, do not exhibit direct antimicrobial activity; they effectively protect mucosal surfaces and prevent microbial colonization. Other ingredients, including xylitol, sorbitol, enzymes, and fluoride, contribute to inhibiting microbial growth. Notably, the addition of core-shell magnetic nanoparticles significantly enhanced the overall antimicrobial activity by generating reactive oxygen species (ROS) that induce oxidative stress and damage microbial cells. These nanoparticles disrupt bacterial energy production by interfering with the electron transport chain, particularly affecting NADH oxidation. Furthermore, they can compromise microbial cell membranes by creating pores or promoting engulfment, ultimately weakening and killing the microbes. Collectively, these mechanisms highlight the effectiveness of magnetic nanoparticles as potent antimicrobial agents [17].

Lysozyme and lactoferrin were also mentioned in reviewed studies. Kang et al. (2017) developed a novel formulation of artificial saliva containing inorganic salts like potassium and sodium chloride, alongside bactericidal components such as lactoperoxidase and potassium thiocyanate, available in both solution and gel forms. Lysozyme in this formulation hydrolyzes Gram-positive bacteria's the cell walls and triggers a nonspecific immune response [19]. While lactoperoxidase lacks direct antimicrobial activity, it generates hypothiocyanite in the presence of thiocyanate and hydrogen peroxide, which exhibits strong bacteriostatic effects [18].

Altin et al. (2021) evaluated the antimicrobial properties of various saliva substitutes containing active ingredients such as lactoferrin and lysozyme from chicken egg whites and breast milk, as well as sodium chloride and magnesium chloride. Lysozyme increases bacterial susceptibility to lysis by hydrolyzing the bondsin the peptidoglycan layer of bacterial cell walls, while lactoferrin contributes to various physiological functions, including antimicrobial and antiviral activities, immunomodulation, and the regulation of cell growth [19].

Murokami et al. (2018) and Murokami et al. (2020) conducted studies mentioning hinokitiol and whey protein as antimicrobial agents in artificial saliva. Murakami et al. (201) investigated antifungal activity of the type of oral moisturizer and the duration of its application by evaluating 17 oral moisturizers: 7 liquids and 10 gels with diverse ingredients. For moisturizers that exhibited growth-inhibitory zones, equal volumes (1:1) of each were combined to

create additional samples. The study identified that the antimicrobial effects were attributed to hinokitiol, protamine, and whey protein. Afterward, Murakami (2020) examined liquid and gel formulations containing antifungal agents such as hinokitiol, whey protein, lactoferrin, and dried egg yolk, with hinokitiol showing the strongest antifungal effect [20,21].

Hinokitiol demonstrates significant antibacterial activity by inhibiting bacterial tricarboxylic acid cycles, while protamine, a protein derived from salmon and herring, is recognized for its broad antimicrobial spectrum. However, caution is recommended with artificial saliva containing whey protein due to the risk of allergic reactions in individuals with a history of milk allergies [21]. Egg yolk antibodies bind specifically to toxins produced by *Candida*, neutralizing their harmful effects. In addition to analyze the active ingredients, the study investigated how temperature and pH differences influence the optimal storage conditions for maintaining antifungal efficacy. After testing and evaluating inhibition zones, results showed that the formulations performed best in conditions that closely mimic natural saliva—specifically, a pH above 6.5 and body temperature (37°C)—when used immediately after opening. These conditions enhanced antifungal effectiveness [20].

The limitations of this study built upon the limited number of articles discussing the antimicrobial components of artificial saliva used for xerostomia therapy. This limited literature restricts the comprehensiveness of the findings and may overlook potential active ingredients that could enhance the therapeutic efficacy of artificial saliva. Additionally, variations in study methodologies and the lack of standardized criteria for evaluating antimicrobial effectiveness further complicate the ability to draw definitive conclusions.

4. Conclusion

Antimicrobial agents used in artificial saliva are xylitol, sorbitol, lysozyme, lactoferrin, lactoperoxidase with potassium thiocyanate, hinokitiol, protamine, whey protein, dried egg yolk, system of enzymes, fluoride ions, hydroxyethylcellulose, and core-shell magnetic nanoparticles. Various other active ingredients may also serve as antimicrobial agents. Further research is needed to continuously enhance the quality of artificial saliva.

Compliance with ethical standards

Disclosure of Conflict of interest

The authors have no conflict of interest to declare.

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