

Antibacterial effects of Nanyang Kiwifruit extract toothpaste on common oral pathogens

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Abstract

Objective Oral diseases exhibit a multifactorial etiology. Plant-derived antimicrobial agents have gained recognition in daily personal care because of their eco-friendly, natural, and biodegradable properties. This study aimed to investigate the antibacterial efficacy of Nanyang kiwifruit extract toothpaste against common oral pathogens, in comparison with four commercially available toothpastes, to identify effective strategies for controlling pathogenic microorganisms and preventing oral diseases.

Methods Five toothpaste formulations (grape probiotic, white, grape-flavored, peach-flavored, and kiwifruit extract) were prepared as gradient dilutions via two-fold serial dilution. Each dilution series was mixed 1:1 with standardized suspensions of *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, or *Proteus vulgaris* (0.5×10^6 CFU/ml), followed by overnight incubation at 37°C. Minimum inhibitory concentrations (MICs) were determined spectrophotometrically at OD600. Minimum bactericidal concentrations (MBCs) were subsequently assessed by plate spreading of 100 µl aliquots of the overnight cultures onto LB agar, with bactericidal activity confirmed as a $\geq 99.9\%$ CFU reduction.

Results The kiwifruit extract toothpaste demonstrated comprehensive antibacterial activity against all four tested pathogens, exhibiting broader-spectrum inhibitory effects compared with commercial toothpastes.

Conclusion The Nanyang kiwifruit extract toothpaste demonstrated broad-spectrum antimicrobial activity against both bacterial and fungal pathogens, exhibiting superior efficacy and broader coverage compared with single-target commercial toothpastes. Its multi-pathogen inhibitory capacity addresses a critical limitation of conventional

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antibacterial toothpastes that primarily target specific microbial subsets, thereby providing greater microbial control for oral ecological balance.

Keywords: antimicrobial toothpaste, kiwifruit extract, MIC, MBC, oral microbiota

Introduction

Oral diseases are prevalent in daily life, significantly impacting quality of life through manifestations such as oral mucosal lesions ^[1], periodontitis ^[2,3], gingival swelling, bleeding, and halitosis, and have become a major global public health issue. The importance of the oral microbiota is increasingly recognized ^[4]. As the gateway to the digestive tract, the oral cavity hosts nearly 700 microbial species, with diversity second only to the gut microbiota ^[5]. These microorganisms colonize the entire oral cavity, including tooth surfaces, the tongue, oral secretions, and saliva. The oral mucosa typically harbors minimal bacterial growth as a result of its defense mechanisms, whereas the tongue and teeth support denser microbial communities. The tongue accumulates oral debris, such as desquamated epithelial cells, leukocytes, and food residues. Bacteria on tooth surfaces secrete viscous extracellular polymeric substances, forming robust networks (biofilms), with dental plaque representing a macroscopic manifestation of these biofilms ^[6]. Dysbiosis of the oral microbiota is closely associated with dental caries, periodontitis, and systemic infections ^[7,8]. *Candida albicans*, a common pathogenic fungus in the oral cavity, forms biofilms that contribute to refractory infections and collaborate with bacteria in the pathogenesis of caries and mucosal infections^[9,10]. *Staphylococcus aureus* can induce angular cheilitis, parotitis, and mucositis ^[11], while *Escherichia coli* is implicated in periodontitis ^[12]. *Proteus vulgaris*, an acid-producing bacterium, may participate in enamel demineralization.

Toothpaste, as a core daily oral hygiene product, enhances mechanical friction during brushing to remove food debris, soft deposits, and dental plaque. The efficacy of its antimicrobial components directly influences the oral microecological balance. With growing demands for oral health, toothpaste formulations have evolved from basic cleansers to functional products incorporating antimicrobial agents. These advanced formulations inhibit bacterial proliferation, thereby reducing the incidence of caries and periodontal diseases. Current commercial toothpastes primarily contain synthetic antimicrobials (e.g., triclosan, domiphen bromide, sodium benzoate, sodium monofluorophosphate, zinc citrate, and sodium lauryl sulfate) or natural plant extracts (e.g., *Lonicera japonica*, *Panax notoginseng*)^[13].

Kiwifruit, renowned as the “King of Fruits,” exhibits diverse health benefits including asthma alleviation, immune enhancement, diabetes management, cancer prevention, skin repair, anemia treatment, and cardiovascular improvement. Rich in carbohydrates, proteins, vitamins, minerals, and polyphenols, kiwifruit extract is recognized for its anti-inflammatory, antimicrobial, anticancer, and anti-aging properties, as well as its ability to mitigate inflammation and oxidative stress induced by airborne particulates¹⁴. In our preliminary study, Nanyang kiwifruit extract was innovatively incorporated into a toothpaste formulation, demonstrating significant promotion of oral mucosal healing¹⁵. This follow-up investigation evaluates the antimicrobial efficacy of the kiwifruit-enriched toothpaste

against common oral pathogens through minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays, compared with commercial counterparts. The study aims to further explore its antimicrobial potential, providing more options for oral disease management and daily protection.

Materials and methods

Experimental materials

The following microbial strains were purchased from Beina Chuanglian Biotechnology Co., Ltd: *Candida albicans* BNCC186382, *Staphylococcus aureus* BNCC186335, *Escherichia coli* BNCC133264, and *Proteus vulgaris* BNCC336633. McFarland turbidity standards (PY23108) were obtained from the same supplier. Culture media, including broth medium, YM broth, and YM agar, were prepared in our laboratory.

Instruments and equipment

Laminar flow cabinets (SW-CJ-1D) were obtained from Suzhou Purification Equipment Co., Ltd; electric thermostatic incubators (LC-HN-50BS) were from Shanghai Lichen Instrument Technology Co., Ltd; and Mini Shakers (MH-1) were from Haimen Qilinbeier Instrument Manufacturing Co., Ltd.

Standard bacterial suspension preparation

Stock cultures of *C. albicans* (BNCC186382), *S. aureus* (BNCC186335), *E. coli* (BNCC133264), and *P. vulgaris* (BNCC336633) were sequentially resuscitated from cryopreservation, inoculated onto YM agar plates, subcultured twice in broth medium, and aerobically cultured at 37°C for 18–24 h. Final suspensions were

standardized to 0.5×10^6 CFU/mL using McFarland turbidity standards (No. PY23108).

Toothpaste dilution preparation

Five toothpaste formulations (kiwifruit extract toothpaste, grape probiotic toothpaste, white toothpaste, grape-flavored toothpaste, and peach-flavored toothpaste) were precisely weighed (50 mg each) and homogenized with 5 mL of physiological saline to obtain 10 mg/mL stock solutions. For gradient dilution, 11 sterile tubes (A0–A10) were sequentially prepared. For example, the kiwifruit toothpaste dilution series was generated as follows, A0: 10 mL LB broth (blank control); A1: 800 μ L stock solution + 7.2 mL LB broth (8 mL total, 1 mg/mL); A2: 4 mL A1 solution + 4 mL LB broth (0.5 mg/mL); subsequent dilutions (A3–A10) were prepared via two-fold serial dilution. Parallel dilution protocols were implemented for other toothpaste types.

Bacterial inoculation

Toothpaste dilutions were sequentially mixed 1:1 (v/v) with standardized bacterial suspensions (0.5×10^6 CFU/mL) according to the inoculation scheme detailed in Table 1. Triplicate experiments (labeled A1-1, A1-2, A1-3) were conducted. Post-inoculation, mixtures were transferred to a 37°C incubator with orbital shaking (160 rpm) for 6–12 h.

MIC determination

The OD600 of the bacterial suspension was measured hourly using a spectrophotometer. The MIC was defined as the lowest toothpaste concentration showing $\geq 90\%$ growth inhibition compared with the A0 blank control (LB broth only). Triplicate values were averaged for the final MIC calculation.

Table 1. Inoculation protocol.

Tube Label	Reagent Type and Volume	Bacterial Suspension from Step 1
A0-1	A0: 1 ml	1 ml
A1-1	A1: 1 ml	1 ml
...	...	...
A11-1	LB: 2 ml	0 ml

MBC determination

From tubes exceeding the MIC values, 100 µl aliquots were spread onto LB agar plates and incubated inverted at 37°C for 24 h. The MBC was identified as the lowest concentration yielding <5 CFU per plate.

Statistical methods

The data were analyzed and organized using GraphPad Prism 7.0 statistical software. Measurement data are expressed as the mean standard deviation (s).

Results

The kiwifruit toothpaste exhibited stronger inhibitory effects against C. albicans than the four commercial toothpaste formulations

MIC values were determined via spectrophotometric measurement of the OD600. Initial experimental groups (grape probiotic toothpaste groups 1–4, white toothpaste groups 1–3, grape-flavored toothpaste groups 1–4, and the first group of peach-flavored/kiwifruit toothpaste) were excluded because of interference from high toothpaste concentrations causing elevated absorbance readings. Lower OD600 values indicated stronger antibacterial efficacy and reduced bacterial growth. As shown in Figure 1, the Nanyang kiwifruit toothpaste demonstrated superior inhibitory activity against *C. albicans* compared with other formulations, with an efficacy ranking as follows: Nanyang kiwifruit toothpaste > peach-flavored toothpaste > grape-fla-

vored toothpaste > white toothpaste > grape probiotic toothpaste.

The kiwifruit toothpaste demonstrated stronger inhibitory effects against S. aureus compared with the other four toothpaste formulations

OD600 measurements were performed after 12 h of treatment of *S. aureus* with toothpaste dilutions. As shown in Figure 2, the Nanyang kiwifruit toothpaste exhibited superior inhibitory efficacy against *S. aureus* compared with other formulations, with an efficacy ranking as follows: Nanyang kiwifruit toothpaste > peach-flavored toothpaste > white toothpaste > grape probiotic toothpaste > grape-flavored toothpaste.

The kiwifruit toothpaste demonstrated superior inhibitory activity against E. coli compared with the four other tested formulations

As shown in Figure 3, the kiwifruit toothpaste exhibited stronger inhibitory effects on *E. coli* growth than the four other formulations. Notably, only the kiwifruit and peach-flavored toothpastes demonstrated inhibition against *E. coli*, while no suppression was observed with grape probiotic, white, or grape-flavored toothpastes.

Inhibitory activity against P. vulgaris was observed exclusively with the kiwifruit toothpaste formulation

As shown in Figure 4, the kiwifruit toothpaste exhib-

ited superior inhibitory activity against *P. vulgaris* compared with the four other formulations. Notably, among all tested toothpastes, only the kiwifruit formulation demonstrated detectable inhibition against

P. vulgaris, while no suppression was observed with grape probiotic toothpaste, white toothpaste, peach-flavored toothpaste, or grape-flavored toothpastes.

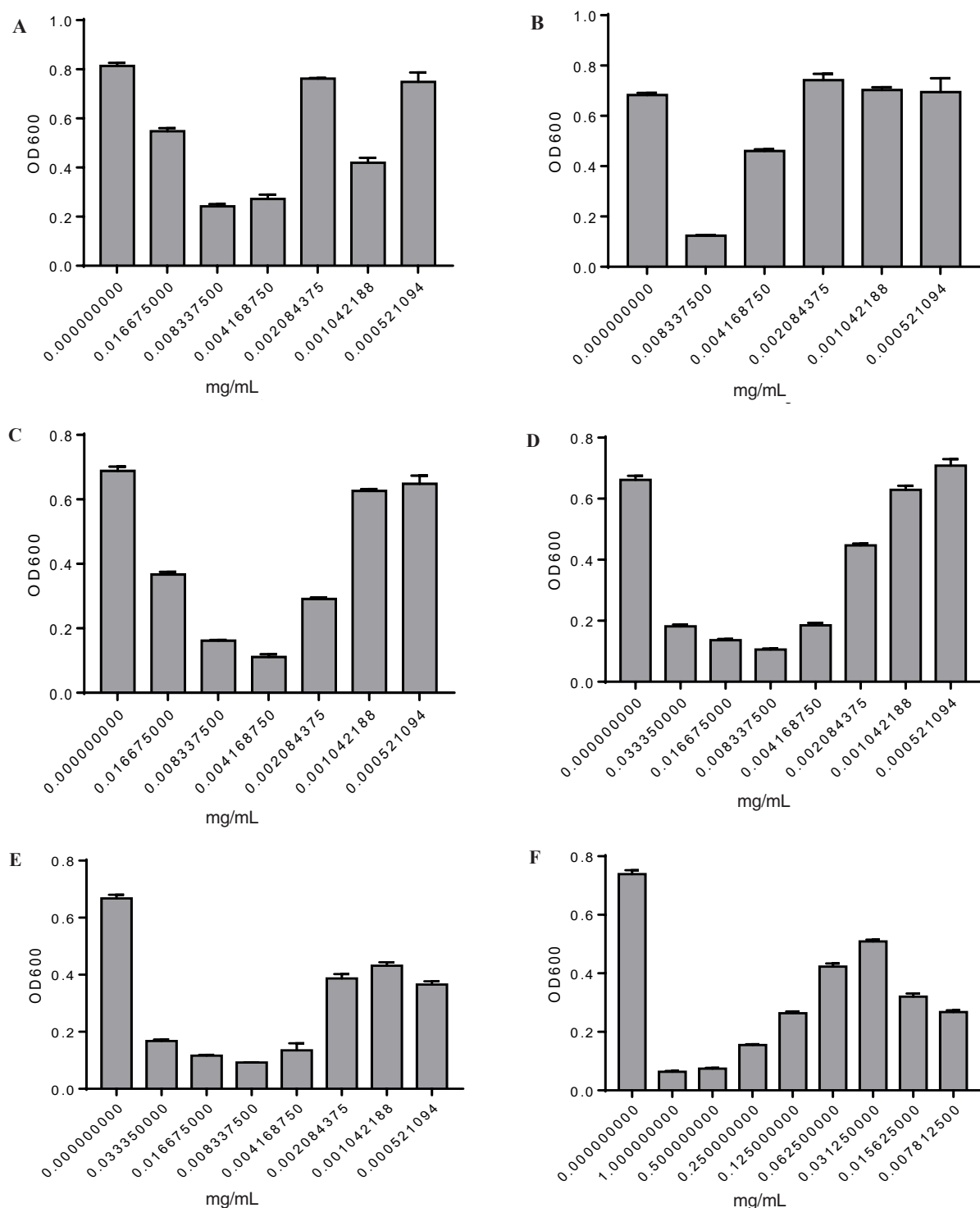


Figure 1. Minimum inhibitory concentration (MIC) of five toothpaste formulations and amphotericin B against *Candida albicans*.

(A) Grape probiotic toothpaste. (B) White toothpaste. (C) Grape-flavored toothpaste. (D) Peach-flavored toothpaste. (E) Nanyang kiwifruit extract toothpaste. (F) Amphotericin B (positive control). Each concentration was tested in triplicate.

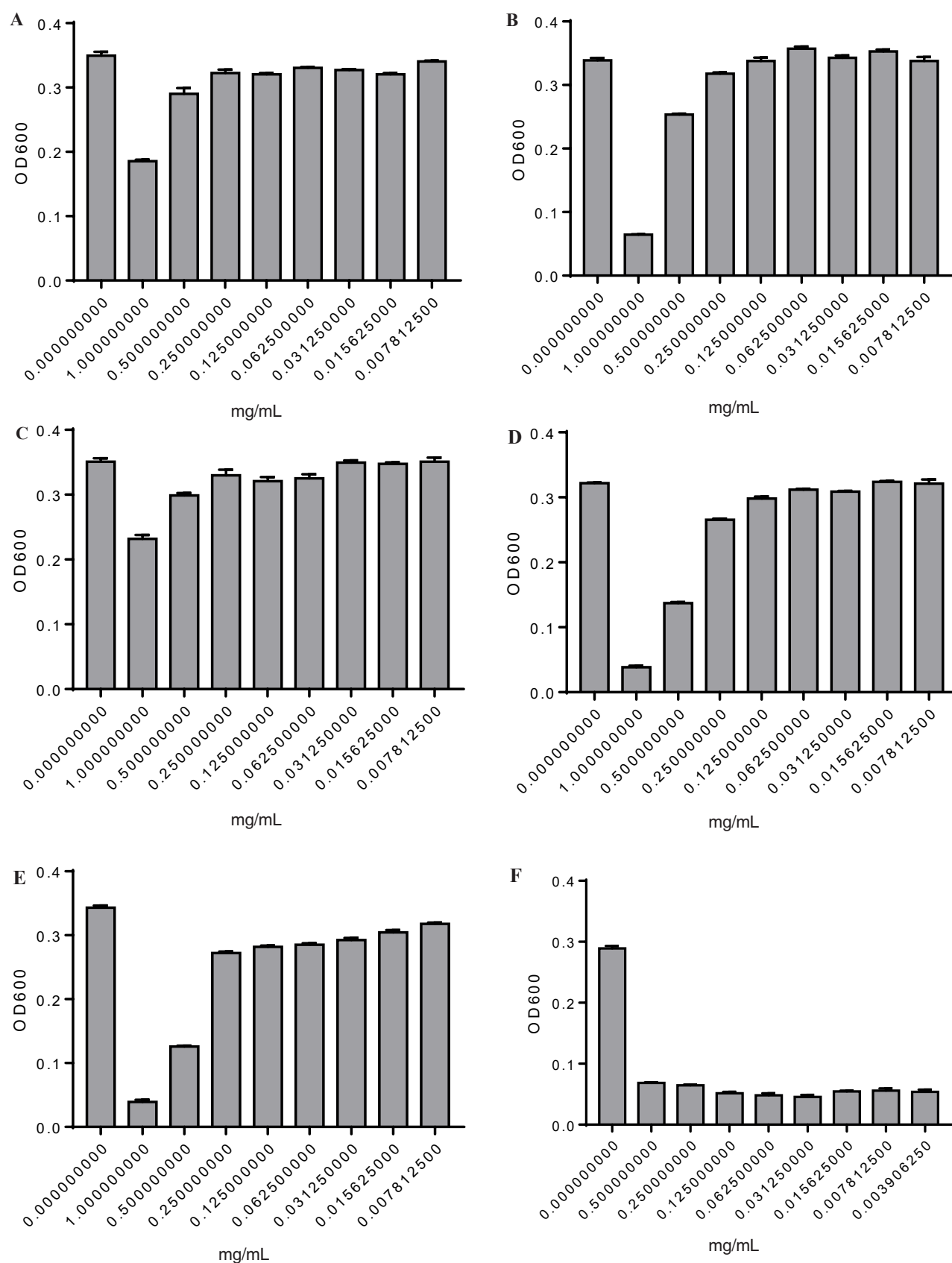


Figure 2. Minimum inhibitory concentration (MIC) of five toothpaste formulations and gentamicin sulfate against *Staphylococcus aureus*.

(A) Grape probiotic toothpaste. (B) White toothpaste. (C) Grape-flavored toothpaste. (D) Peach-flavored toothpaste. (E) Nanyang kiwifruit extract toothpaste. (F) Gentamicin sulfate (positive control). Each concentration was tested in triplicate.

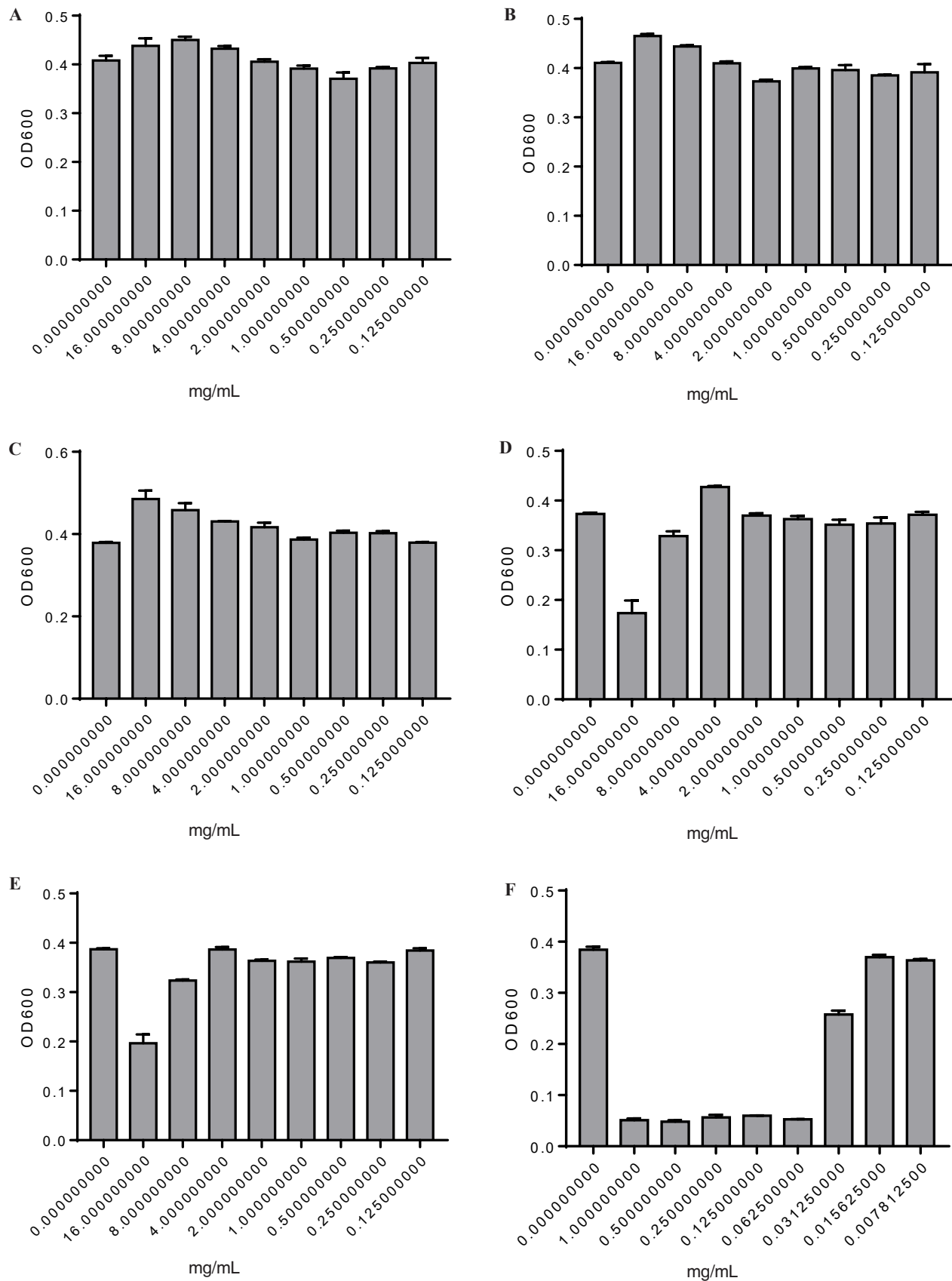


Figure 3. Minimum inhibitory concentration (MIC) of five toothpaste formulations and methicillin against *Escherichia coli*. (A) Grape probiotic toothpaste. (B) White toothpaste. (C) Grape-flavored toothpaste; (D) Peach-flavored toothpaste. (E) Nanyang kiwifruit extract toothpaste. (F) Methicillin (positive control). Each concentration was tested in triplicate.

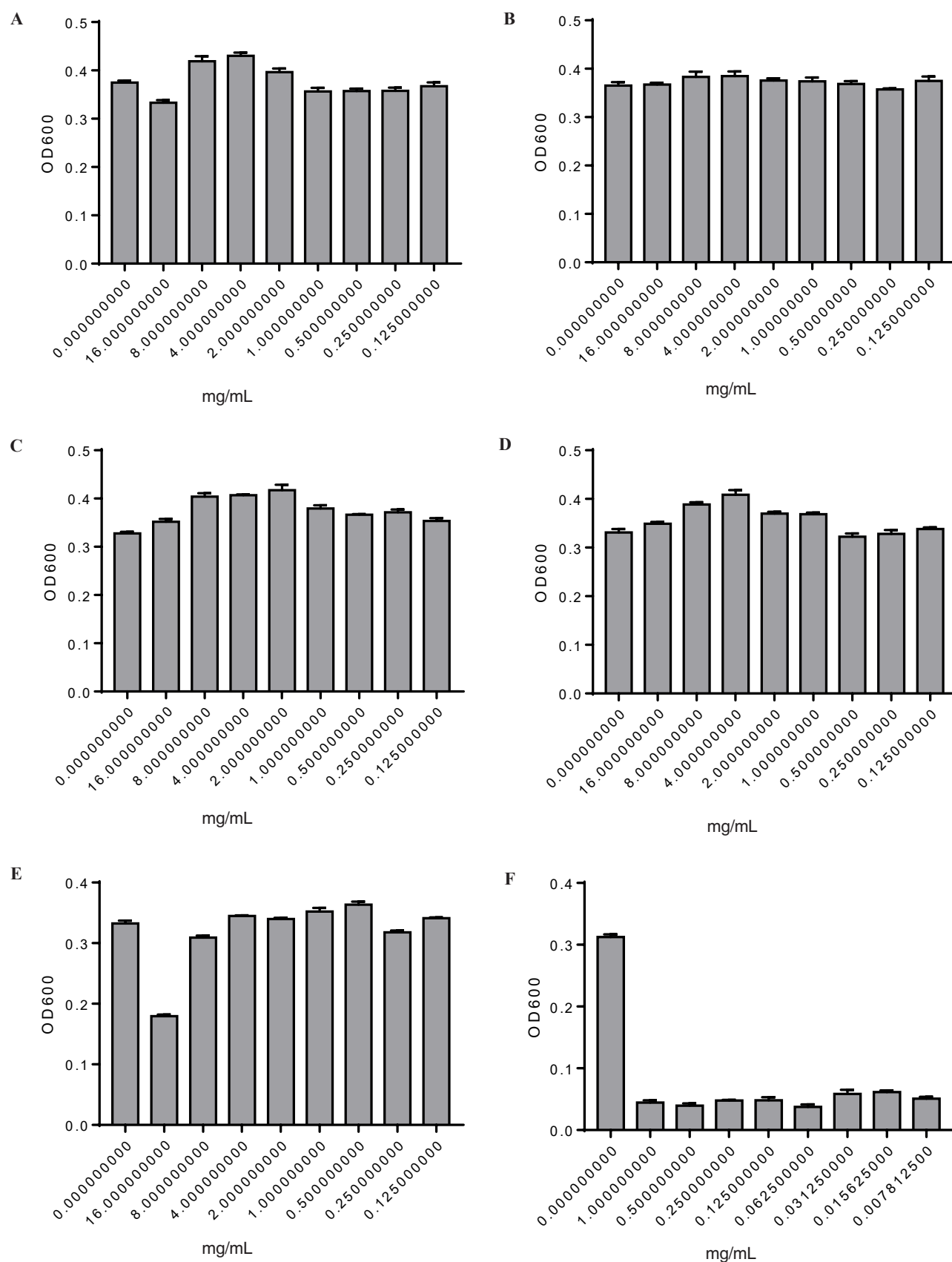


Figure 4. Minimum inhibitory concentration (MIC) of five toothpaste formulations and gentamicin sulfate against *Proteus vulgaris*.

(A) Grape probiotic toothpaste. (B) White toothpaste. (C) Grape-flavored toothpaste; (D) Peach-flavored toothpaste. (E) Nanyang kiwifruit extract toothpaste. (F) Gentamicin sulfate (positive control). Each concentration was tested in triplicate.

The kiwifruit toothpaste demonstrated broad-spectrum antimicrobial efficacy

The MBC values were further assessed via LB plate spreading assays (Table 2). All formulations except white toothpaste exhibited both bacteriostatic and bactericidal effects against *C. albicans*, while all toothpastes showed bactericidal activity against *S. aureus*. Notably, the kiwifruit formulation achieved the lowest MBC values against *C. albicans* (0.0021 mg/mL) and *S. aureus* (0.5 mg/mL), with a significant reduction observed against *E. coli* and detectable activity against *P. vulgaris* (16 mg/mL).

Discussion

This study experimentally validated the antimicrobial efficacy of the developed Nanyang kiwifruit extract toothpaste. Compared with four commercial toothpastes (grape probiotic, white, peach-flavored, and grape-flavored), the kiwifruit formulation exhibited superior inhibitory effects against *C. albicans*, *S. aureus*, *E. coli*, and *P. vulgaris*. Notably, it was the only formulation active against all four pathogens, particularly demonstrating exclusive inhibition of *P. vulgaris*.

Oral health, a critical component of overall wellbeing, relies heavily on effective hygiene practices. As a daily essential, toothpaste formulations have evolved from basic cleaning agents to functionally enriched products. Natural antimicrobial toothpastes, exemplified by the

kiwifruit-based formulation described here, offer advantages including reduced microbial resistance risks, minimized chemical irritants, and maintenance of oral ecological balance, aligning with growing consumer demand for safe and natural oral care solutions¹⁶⁻¹⁸.

Our previous research confirmed the ability of the kiwifruit-based formulation to accelerate oral mucosal healing. The current findings further establish its broad-spectrum antimicrobial properties, suggesting potential benefits for daily oral care in patients with infectious conditions. Future studies should explore additional functionalities such as whitening, gingival bleeding reduction, and breath freshening. Nanyang kiwifruit extract toothpaste addresses the limitations of single-target antimicrobial toothpastes through its broad-spectrum efficacy and natural composition, providing a promising alternative for comprehensive oral health maintenance.

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Table 2. Minimum bactericidal concentrations (MBC, mg/mL) of five toothpastes against common oral pathogens.				
Formulation	<i>C. albicans</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. vulgaris</i>
Grape probiotic toothpaste	0.0042	1	ND	ND
White toothpaste	ND	1	ND	ND
Grape-flavored toothpaste	0.0042	1	ND	ND
Peach-flavored toothpaste	0.0042	1	ND	ND
Kiwifruit extract toothpaste	0.0021	0.5	ND*	16

Notes: ND = Not detected at maximum test concentration (10 mg/ml); * = Significant reduction (≥50% CFU decrease).

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Author Contributions

Experimental operation and article writing: Tao Liu. Data analysis: Hongmei Wang. Guidance on the experimental design: Rui Xiao and Lin Feng. Experimental assistance: Hua Li, Lidan Shi and Panpan Meng.

Conflicts of Interest

All authors declare that there are no conflicts of interest in this article.

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