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In Vitro Antimicrobial Efficacy of *Aloe vera* Gel Against Oral and Skin Pathogens: A Cross-Sectional Laboratory Study

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ABSTRACT:

Background: The accelerating global crisis of antimicrobial resistance (AMR) has renewed interest in botanical antimicrobials as low-cost adjuncts for the management of common oral and skin infections. *Aloe vera* (L.) Burm.f. is one of the most widely cultivated and traditionally used medicinal plants of South India, yet quantitative comparative screening of its gel against clinically relevant oral and cutaneous pathogens remains limited.

Objective: To evaluate the concentration-dependent in vitro antimicrobial efficacy of *Aloe vera* leaf gel against five oral and skin pathogens and to determine its bactericidal potential.

Methods: In this cross-sectional in vitro laboratory comparative study, *Aloe vera* gel was tested against *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* using agar well diffusion at 25, 50, 75 and 100% v/v in triplicate, alongside a standard-antibiotic positive control and a vehicle negative control. Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by broth dilution. The gel was physicochemical and phytochemicals characterized (pH, viscosity, total phenolic and flavonoid content, dry matter). Data were summarized as mean ± SD; the 100% gel was compared with the negative control using independent-samples t-tests with Cohen's d ($p < 0.05$).

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Results: *Aloe vera* gel produced concentration-dependent inhibition against all five pathogens. At 100% v/v, zones ranged from 19.57 ± 0.67 mm (*S. aureus*) to 13.27 ± 0.45 mm (*P. aeruginosa*). Gram-positive organisms were most susceptible and *P. aeruginosa* least. Activity was bactericidal (MBC:MIC ≤ 2) at achievable concentrations, with MIC values of 25–75% v/v. All 100%-versus-control comparisons were highly significant ($p < 0.001$) with very large effect sizes (Cohen's d 21.00–78.30). The gel was mildly acidic (pH 4.64) and rich in phenolics (37.75 mg GAE/g) and flavonoids (19.52 mg QE/g).

Conclusion: *Aloe vera* gel demonstrated bactericidal, dose-dependent antimicrobial activity against oral and skin pathogens, below the standard antibiotic but far exceeding the vehicle.

1. INTRODUCTION

Antimicrobial resistance (AMR) is among the foremost threats to global public health, driving rising treatment failure, prolonged illness and mortality across both community and hospital settings. The relentless emergence of multidrug-resistant Gram-positive and Gram-negative organisms, coupled with a slowing pipeline of novel antibiotics, has intensified the search for complementary and alternative antimicrobial strategies. Plant-derived compounds, refined over millennia of ethnomedical use, offer a chemically diverse and often inexpensive reservoir of antimicrobial molecules that may serve as adjuncts to conventional therapy or as leads for new agents [1,2].

Aloe vera (L.) Burm.f. is a succulent xerophyte whose mucilaginous leaf gel has been used for centuries in traditional systems of medicine for wound healing, skin care and oral hygiene. Its biomedical applications have been reviewed extensively, spanning anti-inflammatory, immunomodulatory, antioxidant, wound-healing and antimicrobial activities [3]. The therapeutic properties of the gel are attributed to a complex matrix of bioactive constituents, including polysaccharides (notably acemannan), anthraquinones such as aloin and emodin, phenolic acids, flavonoids, sterols, saponins and enzymes. Several of these constituents—particularly phenolics and flavonoids—are recognised for their capacity to disrupt microbial membranes, chelate metal ions, inhibit key enzymes and interfere with biofilm formation, providing a plausible mechanistic basis for antimicrobial action [4,5]. The favorable biocompatibility of *Aloe vera* has also made it a popular matrix for tissue-engineering scaffolds, wound dressings and hydrogel composites [6,7].

Oral and skin infections represent a substantial and largely preventable burden of disease worldwide. Dental caries and periodontal disease are driven by biofilm-forming organisms such as *Streptococcus mutans* and *Enterococcus faecalis*, and chemical plaque control remains a cornerstone of prevention [8]. Natural antimicrobials, including plant extracts incorporated into toothpastes and mouth rinses, have shown measurable activity against oral pathogens and are increasingly explored as gentler adjuncts to synthetic agents [9]. On the skin, *Staphylococcus aureus* is the predominant pathogen in pyoderma, wound infection and impetigo, while opportunists such as *Escherichia coli* and *Pseudomonas aeruginosa* complicate chronic wounds, burns and device-associated infection. The topical accessibility of these infections makes them especially attractive targets for botanical antimicrobials that can be applied directly at high local concentrations.

A considerable body of laboratory work supports the antimicrobial potential of *Aloe vera*, much of it involving *Aloe vera*-mediated green synthesis of metallic nanoparticles. Silver, copper-oxide and composite nanomaterials prepared using *Aloe vera* extract have shown broad antibacterial and antifungal activity against skin, enteric and oral organisms [10,11]. However, the intrinsic activity of the native gel itself—free of nanoparticle enhancement—against a panel of clinically relevant oral and skin pathogens, assessed across a graded concentration range with formal bactericidal endpoints, is less systematically documented. Such simple, low-cost screening is highly relevant to South India, where *Aloe vera* is widely cultivated, inexpensive and deeply embedded in traditional medicine and everyday skin and oral care.

The present study therefore aimed to evaluate the concentration-dependent in vitro antimicrobial efficacy of *Aloe vera* leaf gel against five oral and skin pathogens (*S. aureus*, *S. mutans*, *E. faecalis*, *E. coli* and *P. aeruginosa*) using agar well diffusion across 25–100% v/v, to determine minimum inhibitory and bactericidal concentrations, and to relate the observed activity to the phenolic

and flavonoid content of the characterized gel. We hypothesized that *Aloe vera* gel would show dose-dependent, bactericidal activity, with Gram-positive organisms more susceptible than Gram-negative organisms.

2. MATERIALS AND METHODS

2.1 Study design

This was a cross-sectional in vitro laboratory comparative study conducted at the Department of Pharmacognosy/Microbiology medical or dental college, South India, during [v.no.2536]. The study evaluated the antimicrobial activity of *Aloe vera* leaf gel against a defined panel of oral and skin pathogens using standardized agar well diffusion and broth dilution assays.

2.2 *Aloe vera* collection, authentication and gel extraction

Mature, healthy *Aloe vera* (L.) Burm.f. leaves were collected locally in South India, during [v.no.2536]. The plant material was taxonomically authenticated at the [Department of Pharmacognosy/Microbiology], and a voucher specimen ([voucher specimen number]) was deposited for reference. Leaves were washed, surface-disinfected and the outer rind removed; the transparent inner parenchymatous gel (fillet) was excised aseptically, homogenized and used as the test material. The freshly prepared gel was serially diluted with sterile vehicle to obtain working concentrations of 25, 50, 75 and 100% v/v.

2.3 Gel physicochemical and phytochemical characterization

Six independent gel preparations (n = 6) were characterized. pH was measured with a calibrated digital pH meter and viscosity with a rotational viscometer (reported in centipoise, cP). Total phenolic content (TPC) was determined by the Folin–Ciocalteu method and expressed as milligrams of gallic acid equivalents per gram (mg GAE/g); total flavonoid content (TFC) was determined by the aluminum chloride colorimetric method and expressed as milligrams of quercetin equivalents per gram (mg QE/g). Dry matter was determined gravimetrically and expressed as a percentage. Appearance and odor were recorded qualitatively.

2.4 Test organisms

Five pathogens of oral and cutaneous relevance were tested: *Staphylococcus aureus* (skin), *Streptococcus mutans* (oral), *Enterococcus faecalis* (oral), *Escherichia coli* (skin/opportunistic) and *Pseudomonas aeruginosa* (skin/opportunistic). Organisms were maintained as reference strains/clinical isolates with ATCC reference numbers [ATCC/reference strain numbers]. Working cultures were standardized to a 0.5 McFarland turbidity standard prior to inoculation.

2.5 Agar well diffusion assay

Antimicrobial activity was assessed by agar well diffusion. Standardized inoculate were spread uniformly onto Mueller–Hinton agar (supplemented as appropriate for fastidious organisms). Wells were bored aseptically and loaded with *Aloe vera* gel at 25, 50, 75 and 100% v/v. A standard antibiotic ([standard antibiotic used as positive control]) served as the positive control and the sterile vehicle/solvent as the negative control. Plates were incubated under conditions appropriate to each organism, and the diameter of the zone of inhibition was measured in millimetres (mm). All assays were performed in triplicate.

2.6 Minimum inhibitory and bactericidal concentrations

MIC and MBC were determined by broth dilution. The MIC was defined as the lowest concentration (% v/v) preventing visible growth, and the MBC as the lowest concentration yielding no viable growth on subculture. The MBC:MIC ratio was computed for each organism; a ratio of ≤ 2 was interpreted as bactericidal and > 4 as bacteriostatic. Determinations were performed in triplicate.

2.7 Statistical analysis

Zone-of-inhibition data were summarized as mean ± standard deviation (SD) of triplicate measurements. For each pathogen, the zone produced by 100% v/v Aloe Vera gel was compared with the negative control using an independent-samples t-test ($df = 4$), and the magnitude of effect was quantified with Cohen's d. A two-sided p value < 0.05 was considered statistically significant. Analyses were performed using standard statistical software.

3.RESULTS

3.1 Gel physicochemical and phytochemical characterization

The characterized Aloe Vera gel (n = 6) was a clear to pale-green preparation with a mild characteristic odor and mildly acidic pH (4.64 ± 0.10). It showed a viscosity of 4085.17 ± 264.90 cP and a low dry-matter content (1.13 ± 0.08%), consistent with the hydrated, mucilaginous nature of the fillet. Phytochemicals, the gel was rich in phenolic compounds (total phenolic content 37.75 ± 1.94 mg GAE/g) and flavonoids (total flavonoid content 19.52 ± 1.92 mg QE/g), providing a plausible chemical basis for antimicrobial activity (Table 1).

Table 1. Physicochemical and phytochemical characterization of *Aloe* Vera gel (n = 6).

Parameter	Unit	Mean ± SD
pH	pH unit	4.64 ± 0.10
Viscosity	cP	4085.17 ± 264.90
Total phenolic content	mg GAE/g	37.75 ± 1.94
Total flavonoid content	mg QE/g	19.52 ± 1.92
Dry matter	%	1.13 ± 0.08
Appearance	—	Clear to pale-green gel
Odor	—	Characteristic

GAE, gallic acid equivalents; QE, quercetin equivalents; cP, centipoise.

3.2 Concentration-dependent zones of inhibition

Aloe vera gel produced clear, measurable zones of inhibition against all five pathogens, and zone diameters increased consistently with concentration from 25% to 100% v/v (Table 2, Figure 1). At the lowest concentration (25% v/v), zones ranged from 10.40 ± 1.47 mm for *S. aureus* down to 5.80 ± 1.18 mm for *P. aeruginosa*. At 100% v/v, the largest zone was again recorded against *S. aureus* (19.57 ± 0.67 mm), followed by *S. mutans* (18.33 ± 0.96 mm), *E. faecalis* (16.67 ± 0.15 mm), *E. coli* (13.57 ± 0.90 mm) and *P. aeruginosa* (13.27 ± 0.45 mm). Across all organisms the activity of the gel remained well below the standard-antibiotic positive control (18.83–26.90 mm) but was many-fold greater than the negative control, which produced negligible zones (≤ 0.37 mm).

The overall pattern, visualized in Figure 1, was a monotonic, near-linear dose–response for every organism, with the Gram-positive skin and oral pathogens (*S. aureus*, *S. mutans*) occupying the uppermost trajectories and *P. aeruginosa* the lowest.

Table 2. Mean zone of inhibition (mm ± SD) of *Aloe vera* gel by pathogen and concentration, with controls.

Pathogen	25%	50%	75%	100%	Positive control	Negative control
Staphylococcus aureus	10.40 ± 1.47	14.07 ± 0.32	16.70 ± 0.62	19.57 ± 0.67	26.90 ± 0.89	0.27 ± 0.23
Streptococcus mutans	9.33 ± 0.06	12.10 ± 0.69	15.30 ± 0.89	18.33 ± 0.96	25.67 ± 0.46	0.33 ± 0.35
Enterococcus faecalis	7.87 ± 0.40	10.70 ± 0.72	13.07 ± 1.05	16.67 ± 0.15	22.77 ± 0.71	0.37 ± 0.25
Escherichia coli	7.30 ± 0.20	10.80 ± 0.20	12.07 ± 0.71	13.57 ± 0.90	20.57 ± 0.93	0.07 ± 0.12

Pseudomonas aeruginosa	5.80 ± 1.18	8.50 ± 0.98	11.00 ± 0.78	±	13.27 ± 0.45	±	18.83 ± 0.32	0.10 ± 0.10

Values are mean ± SD of triplicate measurements. Units: mm. Positive control = [standard antibiotic used as positive control]; negative control = sterile vehicle.

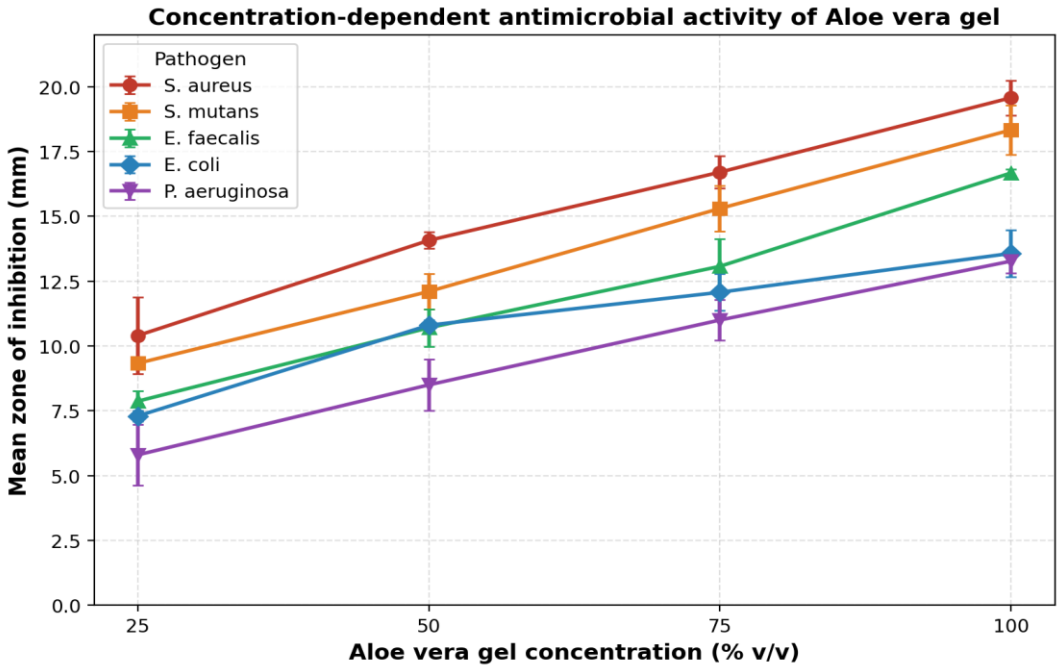

Figure 1. Concentration-dependent mean zone of inhibition (mm) of *Aloe vera* gel against five oral and skin pathogens at 25, 50, 75 and 100% v/v. Error bars represent the standard deviation of triplicate measurements.

3.3 Minimum inhibitory and bactericidal concentrations

Broth dilution confirmed that *Aloe vera* gel was not merely inhibitory but bactericidal against every organism tested, with all MBC:MIC ratios ≤ 2 (Table 3, Figure 2). The most susceptible organisms were the Gram-positive *S. aureus* and *S. mutans* (MIC 25% v/v, MBC 50% v/v), followed by *E. faecalis* and *E. coli* (MIC 50% v/v, MBC 100% v/v). *P. aeruginosa* was the least susceptible, requiring the highest inhibitory concentration (MIC 75% v/v, MBC 100% v/v). The concordance between the diffusion and dilution assays—both ranking *S. aureus* and *S. mutans* most susceptible and *P. aeruginosa* least—reinforces the internal consistency of the observed activity.

Table 3. Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations of *Aloe vera* gel and MBC:MIC ratio by pathogen.

Pathogen	MIC (% v/v)	MBC (% v/v)	MBC:MIC	Interpretation
Staphylococcus aureus	25	50	2.0	Bactericidal
Streptococcus mutans	25	50	2.0	Bactericidal
Enterococcus faecalis	50	100	2.0	Bactericidal
Escherichia coli	50	100	2.0	Bactericidal
Pseudomonas aeruginosa	75	100	≤ 1.3	Bactericidal

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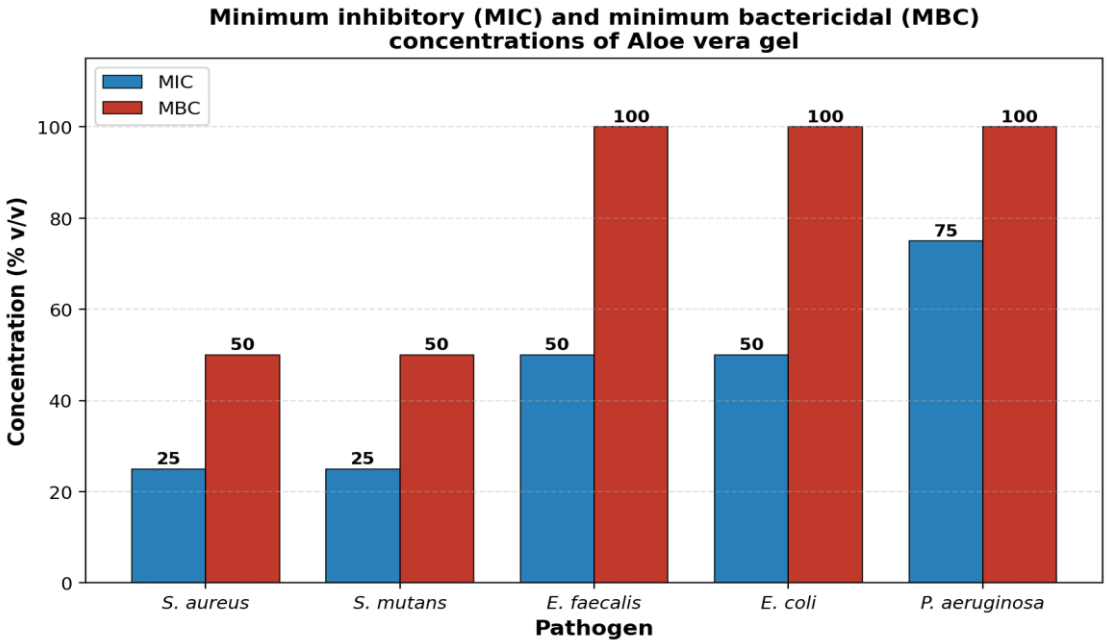

Figure 2. Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (% v/v) of *Aloe vera* gel for each of the five oral and skin pathogens.

3.4 Statistical comparison of 100% gel versus negative control

For every pathogen, the zone produced by 100% v/v *Aloe vera* gel was very highly significantly greater than that of the negative control (independent-samples t-test, df = 4, $p < 0.001$ in all cases), with extremely large effect sizes (Table 4). Cohen's d ranged from 21.00 (*E. coli*) to 78.30 (*E. faecalis*), indicating that the separation between gel and vehicle was many standard deviations wide and far exceeded conventional thresholds for a "large" effect. These results confirm that the observed inhibition reflected genuine antimicrobial activity of the gel rather than any effect of the vehicle.

Table 4. Independent-samples t-test results comparing 100% v/v *Aloe vera* gel with the negative control.

Pathogen	t statistic	df	p value	Cohen's d
Staphylococcus aureus	47.434	4	< 0.001	38.73
Streptococcus mutans	30.474	4	< 0.001	24.88
Enterococcus faecalis	95.901	4	< 0.001	78.30
Escherichia coli	25.718	4	< 0.001	21.00
Pseudomonas aeruginosa	49.375	4	< 0.001	40.31

4. DISCUSSION

This cross-sectional in vitro study demonstrated that *Aloe vera* leaf gel exerts concentration-dependent, bactericidal antimicrobial activity against a panel of clinically relevant oral and skin pathogens. Three findings stand out: a clear dose–response relationship across 25–100% v/v; a consistent susceptibility gradient in which Gram-positive organisms were most susceptible and *Pseudomonas aeruginosa* least; and bactericidal endpoints (MBC:MIC ≤ 2) at concentrations achievable by direct topical or oral application [12,13]. Together, these observations support the plausibility of *Aloe vera* gel as a low-cost adjunct antimicrobial, particularly in resource-conscious settings such as South India.

Dose dependence. The monotonic increase in zone diameter with concentration for every organism (Figure 1) is consistent with a diffusion-limited, dose-driven antimicrobial effect and mirrors the concentration-responsive behavior reported across the *Aloe vera* antimicrobial literature [14-16]. The parallel gradient seen in the MIC/MBC data—lower inhibitory concentrations for the most susceptible organisms—reinforces this interpretation and indicates that the activity is a graded, reproducible property of the gel rather than an all-or-none phenomenon.

Gram-positive versus Gram-negative susceptibility. The greater susceptibility of *S. aureus* and *S. mutans* relative to *E. coli* and especially *P. aeruginosa* accords with a well-recognized structural principle: the thick but single-layered peptidoglycan wall of Gram-positive bacteria is more readily penetrated by phenolic and flavonoid antimicrobials, whereas the outer lipopolysaccharide membrane of Gram-negative organisms acts as an effective permeability barrier. *P. aeruginosa* additionally possesses potent efflux systems and intrinsic resistance mechanisms that make it notoriously refractory to many natural products; its position as the least susceptible organism here is therefore biologically expected and consistent with prior reports on plant extracts and *Aloe vera*-based nanomaterials [11,17]. That the gel nonetheless achieved a bactericidal endpoint against *P. aeruginosa*, albeit only at the highest concentrations, is noteworthy given the clinical difficulty this organism poses in chronic wounds and burns.

Phenolic and flavonoid basis. The characterized gel was rich in phenolic (37.75 mg GAE/g) and flavonoids (19.52 mg QE/g), a chemical profile that provides a coherent mechanistic explanation for the observed activity. Phenolic and flavonoid compounds disrupt cytoplasmic membranes, denature proteins, chelate essential cations, inhibit nucleic acid synthesis and interfere with microbial energy metabolism and biofilm formation [13,18]. The mildly acidic pH (4.64) of the gel may further contribute by creating an unfavorable microenvironment for bacterial growth. These constituents are also the reactive species that drive *Aloe vera*-mediated green synthesis of antibacterial nanoparticles, in which the same reducing phenolic simultaneously cap the metal and contribute intrinsic antimicrobial activity [14,19].

Much of the published work on *Aloe vera* antimicrobial activity has focused on nanoparticle systems—silver, copper-oxide and composite formulations synthesized using *Aloe vera* extract have consistently shown potent broad-spectrum activity against skin, enteric and oral organisms and even antifungal effects against *Candida albicans* [6,7,20]. Composite wound dressings and hydrogels incorporating *Aloe vera* likewise demonstrate antibacterial function alongside favorable release and biocompatibility profiles [8,9,10]. Our findings extend this body of evidence by quantifying the intrinsic activity of the native gel itself, without nanoparticle enhancement, across a graded concentration range with formal bactericidal endpoints. As expected, the native gel was less potent than the standard antibiotic positive control, underscoring that it is best positioned as an adjunct rather than a replacement for conventional agents. In the oral sphere, our results align with reports that natural antimicrobials and plant-based dentifrices inhibit caries-associated organisms such as *S. mutans* and *E. faecalis* and may complement chemical plaque control strategies [16,21].

Oral and skin applications and relevance to South India. The susceptibility of *S. mutans* and *E. faecalis*—key agents of dental caries, biofilm and endodontic infection—supports the exploration of *Aloe vera* gel in mouth rinses, dentifrices and intracranial preparations [22]. The strong activity against *S. aureus*, together with measurable activity against *E. coli* and *P. aeruginosa*, is relevant to topical management of pyoderma, infected wounds and burns, complementing the wound-healing reputation of the gel and the antibacterial composite dressings described in the literature [17]. These applications are especially pertinent to South India, where *Aloe vera* is widely cultivated, inexpensive and firmly established in traditional medicine and everyday skin and oral care; a locally sourced, low-cost antimicrobial adjunct could be valuable in primary-care and community settings confronting rising AMR.

Implications for antimicrobial resistance. As AMR erodes the utility of established antibiotics, adjuncts that reduce antibiotic exposure, target biofilms or act at accessible topical sites are of growing interest. A bactericidal botanical gel that can be applied directly at high local concentration—well above its MIC—may help limit selective pressure while providing symptomatic benefit. Such simple, low-cost screening is a rational first step in prioritising candidate botanicals for more definitive study [23].

5. CONCLUSION

Aloe vera leaf gel demonstrated concentration-dependent, bactericidal antimicrobial activity against all five oral and skin pathogens tested, with Gram-positive organisms most susceptible and *Pseudomonas aeruginosa* least. Activity remained below the

standard-antibiotic positive control but vastly exceeded the vehicle, with very large and highly significant effect sizes, and was bactericidal at achievable concentrations. The gel's substantial phenolic and flavonoid content offers a plausible mechanistic basis.

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