# Evaluation of the antibacterial activity of alum against *Porphyromonas gingivalis*: An *in vitro* study

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**Abstract.** Periodontitis is a globally prevalent chronic inflammatory disease strongly associated with specific periodontal pathogens, particularly *Porphyromonas gingivalis* (*P. gingivalis*), which plays a major role in disease progression. Increasing concerns regarding antibiotic resistance and the adverse effects associated with conventional antiseptics have encouraged the exploration of safe, affordable, and naturally derived antimicrobial alternatives. The present *in vitro* study evaluated the antibacterial efficacy of potassium aluminium sulphate (potash alum) against *P. gingivalis* (ATCC 33277) and determined its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The MIC was assessed using a broth microdilution technique adapted from the Clinical and Laboratory Standards Institute (CLSI) M11 guidelines. Serial two-fold dilutions of potash alum ranging from 100 to 1.56 mg/ml were prepared in supplemented Brain Heart Infusion broth, and bacterial growth was evaluated visually based on turbidity and spectrophotometrically using $OD_{600}$ measurements. The MBC was determined by sub-culturing non-turbid wells onto Brucella blood agar supplemented with hemin and vitamin $K_1$ (BAPHK), followed by anaerobic incubation at 37˚C for 48-72 h. Potash alum demonstrated concentration-dependent antibacterial activity, with an MIC of 12.5 mg/ml (1.25%), exhibiting the complete inhibition of visible growth and a markedly reduced $OD_{600}$ value compared with the positive control. The MBC was identified at 50 mg/ml (5.0%), at which no colonies were detected, corresponding to a reduction of ≥99.9% in viable bacteria. One-way ANOVA followed by Tukey's HSD post-hoc test demonstrated statistically significant differences in $OD_{600}$ values among the treatment groups ($F=338.65$, $P<0.001$). On the whole, these findings indicate that potash alum exhibits measurable inhibitory and bactericidal activity against *P. gingivalis in vitro* and may have potential as an adjunctive antimicrobial agent in periodontal therapy. However, further studies evaluating antibiofilm activity, cytotoxicity, biocompatibility, and clinical efficacy are warranted.

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## Introduction

Periodontitis is a chronic inflammatory disease that, if left untreated, destroys the supporting structures of the teeth, including the periodontal ligament, cementum and alveolar bone. The microorganisms within the biofilm accumulating on the tooth surfaces interact with and are modified by the host immune-inflammatory response (1). In the event that periodontal inflammation is not adequately controlled through appropriate therapeutic interventions, the progressive destruction of the supporting tissues may result in tooth mobility and, eventually, tooth loss. The pathogenesis of periodontitis is multifactorial and involves complex interactions between microorganisms within the subgingival biofilm and host susceptibility factors (2).

A recent analysis of the Global Burden of Disease Study 2021 demonstrated a substantial and increasing global burden of periodontal diseases between 1990 and 2021, with further increases projected by 2040 (3). Earlier global estimates similarly reported that severe periodontitis affected ~11.2% of the global population in 2010, constituting it as one of the most prevalent chronic conditions worldwide (4). The persistent and increasing burden of periodontal disease highlights the need for effective strategies for prevention and management.

*Porphyromonas gingivalis* (*P. gingivalis*) is one of the most well-studied periodontal pathogens associated with chronic periodontitis. It is a Gram-negative, obligate anaerobic, black-pigmented rod and is part of the 'red complex' (5). *P. gingivalis* possesses multiple virulence factors, such as gingipains, fimbriae, lipopolysaccharide, hemagglutinins and capsule-associated components. These virulence factors contribute to colonization, persistence, biofilm formation and the modulation of the host immune-inflammatory response. Gingipains, the major cysteine proteases produced by *P. gingivalis*, can degrade host proteins and interfere with components

of innate immunity, thereby facilitating bacterial persistence and contributing to periodontal tissue destruction. Fimbriae promote adhesion and colonization within the subgingival environment, while lipopolysaccharide and other bacterial components can modulate host inflammatory responses. Through these mechanisms, *P. gingivalis* can contribute to microbial dysbiosis and sustained inflammation, ultimately promoting destruction of periodontal connective tissues and alveolar bone (6). Therefore, the effective control of *P. gingivalis* is a key consideration in the management of periodontal disease.

Scaling and root planing is one of the most widely used procedures in conventional periodontal therapy. However, the complete eradication of pathogenic microorganisms from the deep periodontal pockets and inaccessible sites is often not possible. Adjunctive antimicrobials are therefore used to improve microbial control; yet, each option has limitations. Systemic antibiotics carry the risk of adverse drug reactions and of promoting antimicrobial resistance (7), while chlorhexidine, the most widely used antiseptic, is associated with tooth and tongue staining, altered taste and increased calculus formation that limit its long-term use (8). These drawbacks, together with growing concern about antimicrobial resistance, have stimulated interest in safe, low-cost antimicrobial agents of natural or alternative origin.

Potassium aluminium sulphate dodecahydrate [potash alum; $KAl(SO_4)_2\ 12H_2O$] is an inorganic double salt composed of potassium, aluminium, sulphate and water. It has been used for various purposes, including water purification, wound care, and as an astringent and haemostatic agent in dentistry, as well as in gingival retraction materials. Its astringent action is primarily associated with protein precipitation, which produces tissue contraction and may help control local bleeding. Alum has also demonstrated antimicrobial activity against both Gram- and Gram-negative bacteria. Its antimicrobial effects have been attributed to several mechanisms, including protein precipitation, the disruption of bacterial membrane integrity and the acidification of the surrounding environment, which may interfere with bacterial growth and cellular functions (9).

Although alum has established applications in dentistry and has demonstrated antimicrobial activity against various microorganisms, its antibacterial activity against important periodontal pathogens, particularly *P. gingivalis*, requires further evaluation. Its relatively low cost, availability and established use in oral healthcare provide a rationale for investigating its potential as an adjunctive antimicrobial agent in periodontal therapy. Therefore, the present *in vitro* study was undertaken to evaluate the antibacterial activity of potash alum against *P. gingivalis* (ATCC 33277) by determining its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

## Materials and methods

*Study design*. The present *in vitro* study was conducted at SRM Kattankulathur Dental College and Hospital, Potheri, Tamil Nadu, India, following approval from the Institutional Scientific Committee. The antibacterial activity of potash alum against *P. gingivalis* was evaluated by determining the its MIC and MBC using a broth microdilution method adapted from the Clinical and Laboratory Standards Institute (CLSI) M11 guidelines for anaerobic bacteria (10). The workflow comprised the preparation of potash alum solutions, the standardization of the bacterial inoculum, broth microdilution for MIC determination, and sub-culture of growth-negative wells for MBC determination.

*Culture media*. Brain Heart Infusion (BHI) broth (MilliporeSigma) supplemented with hemin (5 $\mu$g/ml) and vitamin $K_1$ (1 $\mu$g/ml) was used for broth culture and for the broth microdilution assay. Brucella blood agar supplemented with 5% defibrinated sheep blood, hemin (5 $\mu$g/ml) and vitamin $K_1$ (1 $\mu$g/ml), abbreviated BAPHK sourced from Thermo Fisher Scientific, Inc.), was used for revival of the organism and for all sub-cultures, including the MBC determination. The same BAPHK medium was therefore used throughout.

*Test organism and culture conditions*. *P. gingivalis* (ATCC 33277) was sub-cultured on BAPHK plates and incubated anaerobically at 37˚C for 48-72 h in an atmosphere consisting of ~80% $N_2$, 10% $H_2$ and 10% $CO_2$.

*Inoculum preparation*. Following incubation, three to five well-isolated colonies were selected and suspended in sterile saline. The turbidity of the bacterial suspension was adjusted to a 0.5 McFarland standard (~$1.5x10^8$ CFU/ml). The standardized suspension was subsequently diluted in supplemented BHI broth to ~$1x10^6$ CFU/ml; thus, the addition of 100 to 100 $\mu$l of the potash alum dilution yielded a final inoculum of ~$5x10^5$ CFU/ml in each well.

*Preparation of potash alum stock solution*. Analytical-grade potash alum (Anmol Chemicals Pvt. Ltd.) was used, and solutions were freshly prepared prior to each experiment. To create a stock solution of 100 mg/ml, the required amount of potash alum powder was dissolved in sterile distilled water. The prepared solution was sterilized by membrane filtration using a 0.22-$\mu$m syringe filter. To obtain test concentrations, serial two-fold dilutions of the solution were prepared in the supplemented broth medium.

*Determination of MIC*. The determination of MIC was performed using the broth microdilution method in a sterile, flat-bottomed 96-well polystyrene microtiter plates. Two-fold serial dilutions of potash alum were prepared in supplemented BHI broth. A volume of 100 $\mu$l of each potash alum dilution was added to the designated wells, followed by 100 $\mu$l of the standardized *P. gingivalis* inoculum, resulting in a final volume of 200 $\mu$l per well. The tested potash alum concentrations were 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/ml.

Appropriate controls were included on each microtiter plate. The positive growth control consisted of supplemented BHI broth containing the standardized bacterial inoculum without potash alum, whereas the negative sterility control consisted of supplemented BHI broth containing potash alum (100 mg/ml) without bacterial inoculum. The inoculated plates were incubated anaerobically at 37˚C for 48 h in an atmosphere comprising ~80% $N_2$, 10% $H_2$ and 10% $CO_2$. Following incubation, bacterial growth was assessed by visual examination for turbidity and by measuring optical density at 600 nm

Table I. MIC Determination of potash alum against *P. gingivalis* (ATCC 33277) by broth microdilution (n=5).

| Well | Potash alum (mg/ml) | (%) | Growth (visual) | Mean $OD_{600}$ | Interpretation |
|---|---|---|---|---|---|
| 1 | 100.0 | 10.0 | Clear (-) | 0.045 | Inhibited |
| 2 | 50.0 | 5.0 | Clear (-) | 0.048 | Inhibited |
| 3 | 25.0 | 2.5 | Clear (-) | 0.052 | Inhibited |
| 4 | 12.5 | 1.25 | Clear (-) | 0.055 | MIC |
| 5 | 6.25 | 0.625 | Turbid (+) | 0.450 | Growth |
| 6 | 3.12 | 0.312 | Turbid (++) | 0.890 | Growth |
| 7 | 1.56 | 0.156 | Turbid (+++) | 1.120 | Growth |
| PC | Control (broth + bacteria) | 0 | Turbid (+++) | 1.250 | Valid growth control |
| NC | Control (broth + alum) | 10 | Clear (-) | 0.042 | Sterility control |

The symbols in the table indicate the following: -, No visible growth; +, low bacterial growth; ++, moderate bacterial growth; +++, abundant bacterial growth. MIC, minimum inhibitory concentration; OD, optical density at 600 nm; PC, positive control; NC, negative control.

($OD_{600}$) using a Synergy Htx Multimode microplate reader (BioTek Instruments Inc.). The MIC was defined as the lowest concentration of potash alum that completely inhibited visible bacterial growth.

*Determination of MBC*. For the determination of MBC, the wells exhibiting no visible bacterial growth in the MIC assay were selected for sub-culture. An aliquot of 10-50 $\mu$l from each growth-negative well was aseptically removed and spot-inoculated onto freshly prepared supplemented BAPHK plates. The plates were incubated anaerobically at 37˚C for 48-72 h. Following incubation, the number of recovered colonies was recorded. The MBC was defined as the lowest concentration of potash alum producing a ≥99.9% reduction in viable bacteria relative to the initial bacterial inoculum. Thus, concentrations that inhibited visible growth in broth but permitted recovery of viable bacteria on agar were considered inhibitory rather than bactericidal.

The nature of the activity was classified from the MBC:MIC ratio, a ratio of ≤4 being conventionally taken to indicate bactericidal activity and a ratio of >4 bacteriostatic activity (11). As two-fold dilutions were used, MIC and MBC values are accurate to within one dilution step.

*Statistical analysis*. One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test was performed to evaluate differences in $OD_{600}$ values among the concentration groups and controls. A value of $P<0.05$ was considered to indicate a statistically significant difference. The MIC and MBC were determined according to the pre-defined microbiological criteria described above and summarized descriptively. Each concentration group and control included five technical replicate wells (n=5) per experimental run. The experiments were repeated independently, and technical replicates were distinguished from independent experimental repeats during data analysis.

## Results

*MIC*. The results of the broth microdilution MIC assay are summarized Table I and Fig. 1. Potash alum demonstrated a concentration-dependent inhibition of *P. gingivalis* growth. The complete absence of visible turbidity, confirmed by a low mean $OD_{600}$ value of 0.055, was observed at a concentration of 12.5 mg/ml (1.25%), establishing this concentration as the MIC. All wells at concentrations ≥12.5 mg/ml remained visually clear, with $OD_{600}$ values ranging from 0.045 to 0.055, closely approximating the negative sterility control ($OD_{600}$ =0.042). By contrast, wells at sub-inhibitory concentrations (6.25, 3.12 and 1.56 mg/ml) displayed marked turbidity with $OD_{600}$ values of 0.450, 0.890 and 1.120, respectively, progressively approaching the positive growth control ($OD_{600}$=1.250). One-way ANOVA with Tukey's HSD post hoc analysis reported statistically significant differences in $OD_{600}$ values among the test groups ($P<0.001$). The 12.5 mg/ml group did not differ significantly from the negative control ($P>0.05$), whereas the 6.25 mg/ml group exhibited a significantly greater bacterial growth than the negative control ($P<0.001$). The 12.5 mg/ml group also exhibited a significantly lower $OD_{600}$ value than the positive control ($P<0.001$).

*MBC*. The results of MBC determination by sub-culture on BAPHK agar are presented in Table II and Fig. 2. At the MIC concentration (12.5 mg/ml), colony recovery was >50 CFU/plate on sub-culture, indicating a bacteriostatic rather than bactericidal effect at this concentration, with a <3-log reduction from the initial inoculum. At 25 mg/ml (2.5%), a marked reduction in viable bacterial recovery was observed, with 12 colonies recovered, indicating an intermediate bacteriostatic-to-bactericidal effect. The complete eradication of viable organisms (zero colony recovery; ≥99.9% kill) was achieved at 50 mg/ml (5.0%), establishing the MBC at this concentration. No colonies were recovered at 100 mg/ml (10.0%), confirming bactericidal activity at concentrations above the MBC. The MBC:MIC ratio was determined to be 4:1, indicative of a predominantly bacteriostatic effect at the MIC with bactericidal activity at higher concentrations.

## Discussion

Periodontitis is a multifactorial inflammatory disease associated with a complex subgingival microbial community, in

Table II. MBC determination of potash alum against *P. gingivalis* (ATCC 33277) by sub-culture on BAPHK agar.

| Source concentration (mg/m) | Colony count (CFU/plate) | Log reduction | Interpretation |
|---|---|---|---|
| 12.5 (MIC) | >50 colonies | <3 log | Bacteriostatic (growth inhibited in broth; recovery on agar) |
| 25.0 | 12 colonies | ~4 log | Bacteriostatic/weakly bactericidal |
| 50.0 | 0 colonies | >99.9% reduction | MBC (bactericidal) |
| 100.0 | 0 colonies | >99.9% reduction | Bactericidal |

BAPHK, Brucella blood agar supplemented with hemin and vitamin $K_1$; MBC, minimum bactericidal concentration; CFU, colony forming units; MIC, minimum inhibitory concentration.

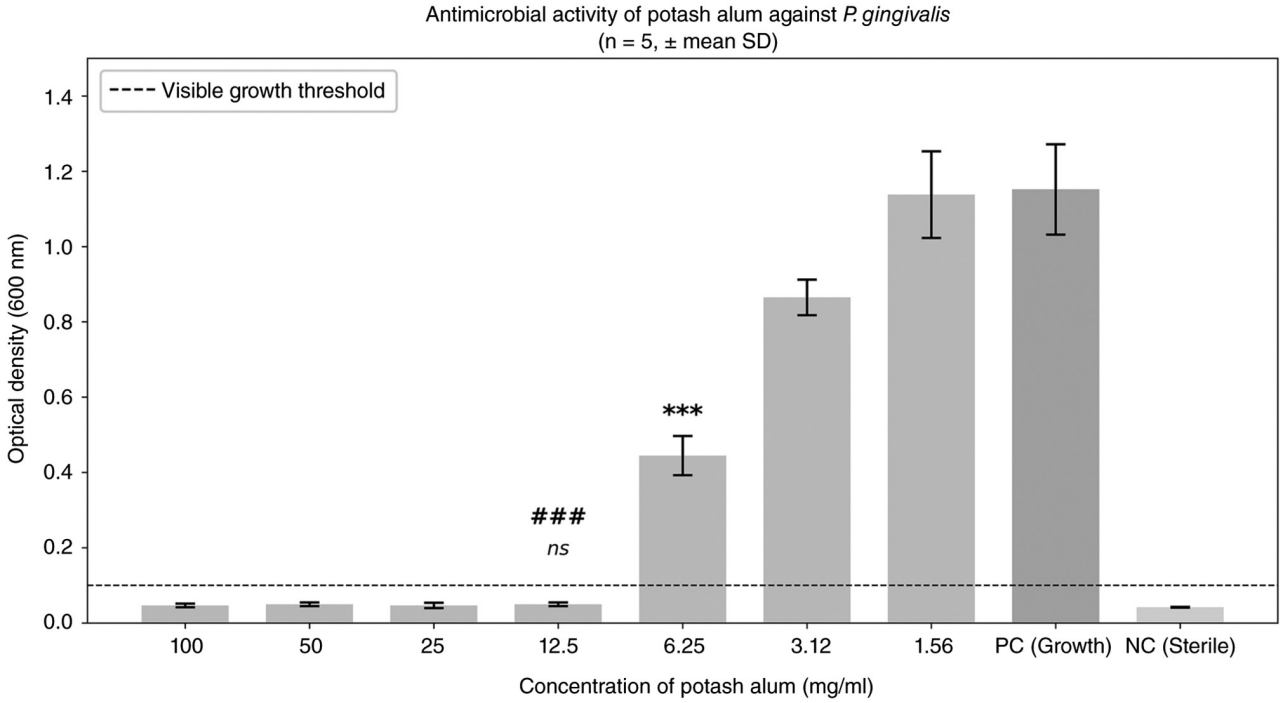



Figure 1. Antimicrobial activity of potassium aluminium sulphate (potash alum) against *Porphyromonas gingivalis* across the concentration gradient. The data demonstrate the concentration-dependent inhibition of bacterial growth, with a minimum inhibitory concentration (MIC) of 12.5 mg/ml (1.25%). The positive control exhibits bacterial growth, whereas the negative control represents the sterile background. $^{***}$P<0.001 vs. NC; ns, not significant vs. NC (P>0.05); $^{###}$P<0.001 vs. PC. PC, positive (growth) control; NC, negative (sterility) control (broth with 100 mg/ml alum, no inoculum).

which microbial dysbiosis contributes to the progressive destruction of the periodontal supporting tissues (2). *P. gingivalis*, a Gram-negative anaerobic bacterium, is considered a keystone periodontal pathogen due to its ability to interact with the microbial community, modulate host immune responses and promote periodontal tissue destruction (12,13). Its major virulence factors, including gingipains and lipopolysaccharides, contribute to the disruption of host tissues and immune homeostasis (14). Although scaling and root planing remains the primary approach for controlling periodontal infection, microorganisms residing within periodontal pockets and biofilms may be difficult to eliminate completely. Consequently, antimicrobial agents used as adjuncts to mechanical periodontal therapy continue to be investigated, particularly those derived from natural or alternative sources.

Potassium aluminium sulphate dodecahydrate [potash alum; $KAl(SO_4)_2$ $12H_2O$] possesses astringent and antimicrobial properties and has historically been used in several medical, dental and industrial applications. The antibacterial properties of alum have been previously described, and the contribution of the present study lies in the type of data it provides, rather than in the observation that alum is antibacterial. Jawad and Auwaid (15) evaluated alum mouthwash as an adjunct in chronic periodontitis using clinical periodontal parameters, without strain-specific MIC or MBC values (15). Shukla *et al* (16) tested a multi-ingredient toothpaste containing alum with zinc citrate, sodium fluoride and xylitol against several oral microorganisms, including *P. gingivalis*. As activity could not be attributed to alum alone, their MIC and MBC values cannot be read as the intrinsic activity of alum (16). Al-Hamdoni and Al-Rawi (17) reported inhibitory (MIC) values for alum against clinical isolates of the red-complex pathogens, while comparing susceptibility-testing methods. By contrast, the present study evaluated potash alum as a single agent against a

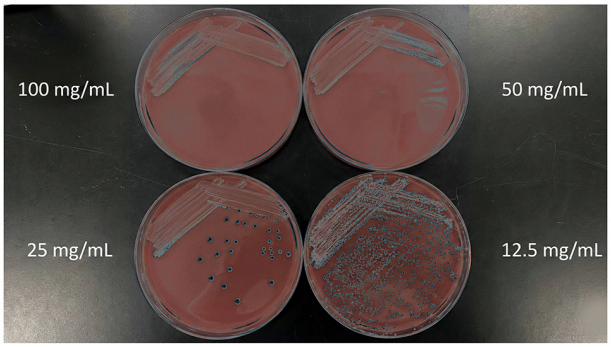



Figure 2. Representative sub-culture plates for the determination of the MBC of potash alum against *Porphyromonas gingivalis*. Following broth microdilution, aliquots from wells showing no visible growth were sub-cultured onto Brucella blood agar supplemented with hemin and vitamin $K_1$. Extensive colony formation was observed at 12.5 mg/ml (minimum inhibitory concentration), whereas markedly reduced colony recovery was observed at 25 mg/ml. No colony growth was observed at 50 or 100 mg/ml, establishing 50 mg/ml as the MBC. MBC, minimum bactericidal concentration.

defined reference strain, determined both MIC and MBC under standardized anaerobic conditions, and used the MBC:MIC association to characterize the nature of its activity. To the best of our knowledge, such combined MIC and MBC data for alum against *P. gingivalis* (ATCC 33277) have not been previously reported, and they provide a quantitative baseline for later formulation, antibiofilm, cytotoxicity and clinical studies.

Several mechanisms underlying the antimicrobial effects of alum have been proposed. Aluminium ions released from alum may interact with bacterial cellular components and proteins, potentially resulting in protein denaturation or precipitation and disruption of cellular functions. The astringent properties of alum may additionally contribute to alterations in the local environment and bacterial surface structures. The dissociation of alum in aqueous solution can also influence the surrounding pH, which may interfere with bacterial metabolic processes (18-20). Therefore, the antimicrobial effect of alum is likely to involve more than a single mechanism. However, the present study did not directly investigate these mechanisms, and further mechanistic studies are required to determine their specific contribution to the observed activity against *P. gingivalis*.

Recent investigations of natural and alternative antimicrobial products provide useful context for the present findings. Ali (21) reported synergistic antibacterial interactions between alum and antibiotics against several microorganisms, supporting the potential of alum as an antimicrobial agent and suggesting that its activity may be enhanced when used in combination with conventional antimicrobial agents. Recently, Sunil *et al* (22) investigated aged garlic extract against oral bacteria and evaluated antimicrobial, antibiofilm, cytotoxicity and substantivity properties. Their findings highlight the importance of assessing not only antimicrobial efficacy, but also biofilm activity, tissue compatibility and persistence at the treatment site when considering natural or alternative antimicrobial agents for periodontal applications (22). Nuraini *et al* (23) tested fractionated ethanolic red ginger extract against *Aggregatibacter actinomycetemcomitans* and *P. gingivalis* using *in silico* and *in vitro* approaches, demonstrating continued interest in natural products against periodontal pathogens. On a mass basis, however, alum is considerably less potent than several such agents: For example, allicin has been reported to have an MIC of 4 mg/l and an MBC of 8 mg/l against *P. gingivalis* (24), several orders of magnitude below the concentrations observed herein. Collectively, these studies support the exploration of natural and alternative antimicrobial agents as potential adjuncts to conventional periodontal therapy. Direct comparison across studies is limited by differences in strains, media and endpoints. The advantages of alum are its low cost, availability, chemically defined composition and a history of use in oral care, which may favour topical delivery at relatively high concentration rather than dilute use (15,18).

The findings of the present study should nevertheless be interpreted in the context of its experimental design. The present study was specifically designed to determine the MIC and MBC of potash alum against a single periodontal pathogen under controlled *in vitro* conditions. Therefore, the inhibitory and bactericidal concentrations observed in the present study cannot be directly extrapolated to clinical effectiveness. In particular, the present study does not establish whether the concentrations identified can be safely delivered and maintained within periodontal pockets. Factors such as dilution by gingival crevicular fluid and saliva, interaction with organic material, contact time, substantivity and formulation characteristics may influence antimicrobial activity under clinical conditions.

From a translational perspective, potash alum may have potential for incorporation into locally delivered periodontal formulations, particularly where localized delivery could provide higher antimicrobial concentrations at the site of infection. However, the present MIC and MBC findings alone are insufficient to establish an appropriate therapeutic concentration or formulation. Future studies are therefore required to evaluate the release characteristics, stability, substantivity and tissue compatibility of alum-containing formulations. Cytotoxicity and biocompatibility studies using relevant gingival and periodontal cells are also necessary before clinical application can be considered.

The present study has certain limitations which should be mentioned. The present study was an *in vitro* study of planktonic cells of a single reference strain, and clinical isolates may differ in susceptibility. Biofilm, polymicrobial and host-cell models were not included. No reference antimicrobial, such as chlorhexidine was tested alongside alum; thus, its relative potency cannot be judged. The pH value of the alum dilutions was not controlled and no pH-matched control was included, so the contributions of acidity and of aluminium ions to the effect cannot be separated. The two-fold dilution scheme limits the resolution of MIC and MBC, and the MBC was based on colony counts from subculture aliquots with a defined detection limit rather than time-kill kinetics. The findings cannot be extrapolated directly to clinical effectiveness, since dilution by crevicular fluid and saliva, interaction with organic material, contact time and formulation will all influence activity *in vivo*.

In conclusion, within the limitations of the present *in vitro* study, potash alum inhibited *P. gingivalis* (ATCC 33277) at 12.5 mg/ml (MIC) and was bactericidal at 50 mg/ml (MBC), a four-fold higher concentration. Activity at the MIC appears to be growth-inhibitory rather than killing. These planktonic-cell data provide a quantitative baseline for alum; however, alone, they do not support clinical use. Antibiofilm activity, cytotoxicity, delivery formulation and clinical efficacy need to be

studied before alum can be considered as an adjunct to periodontal therapy.

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**Availability of data and materials**

The data generated in the present study may be requested from the corresponding author.

**Authors' contributions**

NN wrote the original draft of the manuscript, and conducted the literature search. PBR and PLRS conceptualized and designed the study. SB performed the literature review. NN and SB conducted the experiments. NN and PBR contributed to data acquisition and data analysis. AVS supervised the study and contributed to statistical analysis and manuscript reviewing. KR conducted the literature review, contributed to the statistical analysis and revised the manuscript. All authors have read and approved the final version of the manuscript. NN and PBR confirm the authenticity of all the raw data.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Use of artificial intelligence tools**

During the preparation of this work, ChatGPT (OpenAI, https://chatgpt.com/) AI tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript

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