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Original Research Article

In Vitro Antimicrobial Activity of Lyophilized Curcumin Against Porphyromonas gingivalis: A Broth Microdilution Study

Article History:

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Abstract: ***Background:*** Curcumin, the main bioactive compound of Curcuma longa L, exhibits broad-spectrum of therapeutic benefits such as antioxidant, anti-inflammatory, antimicrobial and wound healing effects, followed by its unique ability to target both the microbial and host mediated aspects of periodontal disease. ***Aim:*** The study aimed to compare the potential antimicrobial activity of Lyophilized Curcumin and Chlorhexidine gluconate (CHX) against Porphyromonas gingivalis. ***Material and Methods:*** Brain Heart Infusion broth (BHI) was used as culture medium to evaluate antimicrobial activity against Porphyromonas gingivalis (ATCC 33277) through microdilution assay in 96 well microtiter plates under anaerobic conditions. A two-fold serial dilution of lyophilized curcumin (1 mg/ml) and 2% chlorhexidine was prepared across dilutions D1-D10 and minimum inhibitory concentration (MIC) was determined after incubation at 37°C for 48 h, using optical density measurements (600 nm). Statistical analysis was performed using Kruskal-Wallis & Mann -Whitney U Test with $p<0.05$ considered significant. ***Results:*** Both lyophilized curcumin and 2% chlorhexidine showed concentration dependent inhibition against Porphyromonas gingivalis, CHX exhibited greater potency (MIC at D8 90%, while curcumin showed significant activity (MIC at D7 70% inhibition. Conclusions: Lyophilized curcumin demonstrated significant antimicrobial activity against Porphyromonas gingivalis, although chlorhexidine showed greater inhibitory potency. These findings suggest that curcumin may serve as promising natural adjunct for periodontal therapy.

Keywords: Curcumin, Porphyromonas gingivalis, Chlorhexidine gluconate, broth microdilution, minimum inhibitory concentration.

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INTRODUCTION

Periodontal disease is significant public health problem and is the common reason of tooth loss in adults worldwide.[1] Periodontitis is an infectious disease that causes inflammation of supporting tissues of the teeth, leading to progressive attachment loss, bone loss ,pocket formation or gingiva recession.[2,3] Dental plaque biofilm and its associated microorganisms are primary etiologic with Porphyromonas gingivalis considered one of the key periodontal pathogen.[4,5] Chlorhexidine gluconate which is widely used as antimicrobial agent in periodontal therapy,[6,7] but due to several side effects which led to grow more interest in the herbal alternatives like curcumin , propolis, lemongrass, neem. [8-10] Among these curcumin which is a primary ingredient of turmeric extract belong to (Zingiberaceae family) & is a rhizome, of Curcuma longa L that exhibits broad-spectrum therapeutic effects, including antioxidant, anti-biofilm ,anti-inflammatory, antimicrobial, and wound-healing properties.[11-13] It also has significant anti-inflammatory effects by suppressing COX-2, lipoxygenase, and iNOS , while also downregulating TNF-α, IL-6, and IL-1β , this reduces gingival inflammation, preventing tissue breakdown, and promotes healing.[14] Curcumin's antioxidant capacity helps neutralize ROS and protect tissues from oxidative stress ,while its wound-healing effects enhance fibroblast proliferation, collagen synthesis, and angiogenesis.[15,16] Although several studies have investigated curcumin's antibacterial properties, more comprehensive data is needed to understand its effects on different strains of microorganisms. Thus, the present in vitro study aims at comparing the potential antimicrobial activity of Lyophilized Curcumin and Chlorhexidine gluconate (CHX) against Porphyromonas gingivalis the most potent periodontal pathogen of oral cavity.

MATERIALS AND METHODS

An in-vitro study evaluated the antimicrobial efficacy of Lyophilized curcumin and chlorhexidine gluconate (CHX) against P. gingivalis (ATCC 33277). The study was conducted under standard laboratory conditions at Taq-Gene Pathology Laboratory, Dehradun, using the microdilution method in 96-well U-bottom microtiter plates to determine the Minimum Inhibitory Concentration (MIC) of both agents.

Armamentarium

Porphyromonas gingivalis (ATCC 33277), lyophilized curcumin, and 2% chlorhexidine gluconate (CHX) were used in the investigation. Test solutions and bacterial cultures were prepared using dimethyl sulfoxide (DMSO), distilled water, and Brain Heart Infusion (BHI) broth (HiMedia Laboratories, India). Antimicrobial testing conducted under aseptic circumstances using standard microbiological equipment such as an anaerobic jar, an incubator (37 °C), micropipettes, sterile 96-well U-bottom microtiter plates, a spectrophotometer, an ELISA reader (600 nm), and a laminar airflow cabinet.

Study Design

The present in-vitro study was performed, compared the antimicrobial activity of two test agents against Porphyromonas gingivalis.

Group 1: Lyophilized Curcumin: Using repeated dilutions, curcumin's ability to suppress P. gingivalis growth was assessed.
Group 2: Chlorhexidine gluconate (CHX): CHX served as the standard antibacterial agent and underwent comparable testing to curcumin.
Additionally, a solvent control (DMSO) was used to ensure that the solvent itself did not affect the bacterial growth, however it was used only for validation of the methodology.

Preparation of Fresh Culture

A pure fresh culture of Porphyromonas gingivalis (ATCC 33277) was inoculated into Brain Heart Infusion (BHI) broth which is incubated under anaerobic conditions by using McIntosh and Fildes anaerobic jar at 37° C. To provide a consistent inoculum, the bacterial suspension was standardized to 0.5 McFarland turbidity (1–2 x 10^2 CFU/ml).

Preparation of the Test Agents

The positive control, 2% chlorhexidine gluconate (Neelkand Pharmaceuticals, India), was serially diluted with sterile distilled water. To create a workable concentrations, lyophilized curcumin, a freeze-dried powered extract of Curcumin longa obtained from Soxhlet extraction and followed by lyophilization, was dissolved in dimethyl sulfoxide (DMSO) and then further diluted in BHI broth. As a solvent control, the final DMSO concentration was kept below 1%.

Microdilution assay for broth

By using sterile 96-well U- bottom plates, the microdilution assay was conducted. Total ten concentrations of each test agent (lyophilized curcumin and chlorhexidine gluconate (CHX) were evaluated in the triplicate. Each plate contain suitable controls: a growth control (Medium+Inoculum), a sterile control (medium only), and a solvent control (medium+ inoculum+ DMSO) to exclude growth inhibition caused by solvent.

Addition of broth

The procedure began with the addition of 100 µL of sterile Brain Heart Infusion (BHI) broth into every well of the experiment. This step prevented the formation of air bubbles because they needed to achieve two goals which included precise measurement of optical density and preservation of strict anaerobic conditions needed by P. gingivalis.

Serial dilution of the test agents:

To establish the concentration gradient, the first well received 200µl of the stock solution of either lyophilized curcumin(1mg/ml) or 2% chlorhexidine. Followed by two- fold serial dilution which was performed across the wells up to 10^{th} well, so that each well contained half the concentration of the previous well.

Inoculation with Porphyromonas gingivalis:

Once the dilution series was prepared ,10 µl of standardized P.gingivalis suspension (0.5 McFarland) was inoculated into each well except the sterile control wells, resulting in a final volume of 100 µl per well. The content of each well were gently mixed by pipetting to ensure the uniform, bacterial distribution and consistent inoculum, which is essential for reproducible determination of the antimicrobial activity.

Incubation

After inoculation, the 96-well microtiter plate was covered with sterile lid to prevent contamination and evaporation, and placing the plates inside a McIntosh and Fildes' anaerobic jar to create an oxygen free environment which is required for Porphyromonas gingivalis. The plate was incubated at 37 C for 48h to allow optimal bacterial growth and assessment of antimicrobial activity.

Observation of Growth

The assessment of the bacterial growth appeared after the incubation period, when all the wells received visual inspection. The turbidity wells showed the active P. gingivalis growth while the clear wells exhibited antimicrobial agent effectiveness through growth inhibition. The growth control wells were inspected to confirm that bacterial proliferation occurred under normal condition, followed by sterile control wells were checked to verify the absence of contamination. So this visual assessment delivered an initial qualitative evaluation of antimicrobial effectiveness which served as preliminary validation before the quantitative assessment.

Confirmation using ELISA Reader

The ELISA reader measured the optical density (OD) of each well at the wavelength of 600 nanometres. The MIC was defined as the lowest concentration with OD values comparable to the sterile control, indicating complete inhibition of growth. All measurements were performed in triplicate, and the mean OD values were calculated to improve reliability. These values were then used to assess how effectively lyophilized curcumin and chlorhexidine gluconate (CHX) worked as antimicrobial.

RESULTS

The study evaluated the antimicrobial effects of three substances which include lyophilized curcumin and chlorhexidine gluconate and DMSO as a solvent control (negative control) on the Porphyromonas gingivalis bacterium through broth microdilution testing which used serial dilutions from D1 to D10 with D1 showing the maximum concentration. The researchers determined growth inhibition by measuring optical density (OD) at 600 nanometres. The study found that all groups showed different antimicrobial activities which extended from D1 to D8 according to the Kruskal-Wallis test results which showed statistical significance with a p value less than 0.001.

Chlorhexidine displayed the strongest inhibitory effects which reached approximately 90 percent while lyophilized curcumin achieved about 70 percent inhibition which proved more effective than the solvent control with a p value below 0.05. The results revealed no significant differences between the two groups because all data points at D9 and D10 showed p values above 0.05 which demonstrated that lower concentrations lost their effectiveness. The study found that the minimum inhibitory concentration (MIC) for chlorhexidine occurred at D8 while D7 showed the minimum inhibitory concentration for lyophilized curcumin which demonstrated stronger antibacterial effects against P. gingivalis. The research provides Table 1 and Figure 1 as visual data to show the comparative MIC values and inhibition patterns between the two testing methods.

Common kitchen spices possess properties that may improve oral health, a key component of overall well-being. For example, turmeric contains curcumin, a natural, bioactive component. [17,18] This in-vitro study compared the antimicrobial efficacy of lyophilized curcumin and chlorhexidine gluconate (CHX) against Porphyromonas gingivalis using the broth microdilution method. Both agents inhibited bacterial growth in a concentration-dependent manner; however, chlorhexidine demonstrated superior antimicrobial efficacy. Chlorhexidine inhibited approximately 90% of bacterial growth, while lyophilized curcumin inhibited about 70%, indicating meaningful, but comparatively lower, antibacterial activity. Chlorhexidine's potent inhibitory effect likely stems from its broad-spectrum antimicrobial activity. This, combined with its high substantivity and ability to disrupt bacterial cell membranes, leads to the leakage of intracellular components and, ultimately, bacterial cell death.[19] Although chlorhexidine is effective, prolonged use can cause side effects like tooth staining, altered taste, mucosal irritation, and calculus formation. This has prompted the search for safer, natural alternatives. [19,20] Lyophilized curcumin demonstrated significant antimicrobial activity against P. gingivalis, consistent with previous studies. Curcumin exhibits antibacterial effects through several mechanisms, including disrupting bacterial membranes, inhibiting enzyme systems, interfering with nucleic acid synthesis, and suppressing virulence factors like gingipains.[16,21] Previous studies by Izui et al.[22], Siddhartha et al.[3], and Chaudhari et al.[23] Sha, Balkees Garib, et al.[24] has also demonstrated antibacterial effects against periodontal pathogens, including P. gingivalis, exhibiting low MIC and MBC values and favorable tissue compatibility, thus supporting its potential as a therapeutic adjunct. Mechanistic studies by Murai et al. [25] further suggest that curcumin induces a nutrient-starvation–like state in P. gingivalis, impairing bacterial metabolism and growth. However, several investigations, including studies by Najafi et al. and Jalaluddin et al., have reported stronger antimicrobial activity of chlorhexidine compared with curcumin, which is consistent with the findings of this study. [7,26] The comparatively lower efficacy of curcumin may be related to its poor aqueous solubility and limited bioavailability, although formulation strategies such as nanoparticles or sustained-release systems may enhance its antimicrobial potential. [27] To ensure a stable and standardized preparation of the active compound for accurate evaluation of its intrinsic antimicrobial activity, this study employed lyophilized curcumin. However, the investigation was limited to in vitro conditions and a single bacterial species, failing to account for host factors or the complexity of multispecies biofilms. Despite these limitations, the findings indicate that chlorhexidine remains more potent against P. gingivalis, although lyophilized curcumin exhibits significant, concentration-dependent antibacterial activity. Therefore, curcumin may serve as a potential adjunct in periodontal therapy, particularly given its antimicrobial, anti-inflammatory, and antioxidant properties.[26] Further studies utilizing biofilm models, improved formulations, and clinical trials are required to fully clarify its therapeutic role.

Table 1. Antimicrobial activity of lyophilized curcumin, chlorhexidine gluconate (CHX), and DMSO against Porphyromonas gingivalis across serial dilutions (D1–D10).

Dilution	Curcumin (Mean ± SD)	2% CHX (Mean ± SD)	DMSO (Mean ± SD)	p-value (Kruskal–Wallis)	Significance
D1	0.25067 ± 0.01290	0.07500 ± 0.00625	0.83067 ± 0.00208	<0.001	Significant
D2	0.32700 ± 0.01646	0.12167 ± 0.01504	0.83200 ± 0.00100	<0.001	Significant
D3	0.36433 ± 0.02203	0.27767 ± 0.01097	0.83200 ± 0.00436	<0.001	Significant
D4	0.42733 ± 0.02113	0.37367 ± 0.01554	0.83267 ± 0.00379	<0.001	Significant
D5	0.59233 ± 0.00451	0.47367 ± 0.00862	0.83867 ± 0.00153	<0.001	Significant
D6	0.67033 ± 0.01060	0.57700 ± 0.01646	0.83867 ± 0.00252	<0.001	Significant
D7	0.70700 ± 0.00361	0.63267 ± 0.01069	0.83933 ±	<0.001	Significant

			0.00116		
D8	0.82233 ± 0.00777	0.75533 ± 0.01097	0.84033 ± 0.00208	<0.001	Significant
D9	0.82967 ± 0.00252	0.81567 ± 0.03617	0.84133 ± 0.00208	0.384	Not significant
D10	0.84533 ± 0.00306	0.88333 ± 0.03821	0.84367 ± 0.00252	0.121	Not significant
Values are expressed as Mean ± SD. Intergroup comparisons were performed using the Kruskal-Wallis test. Statistical significance was defined as $p < 0.05$.					

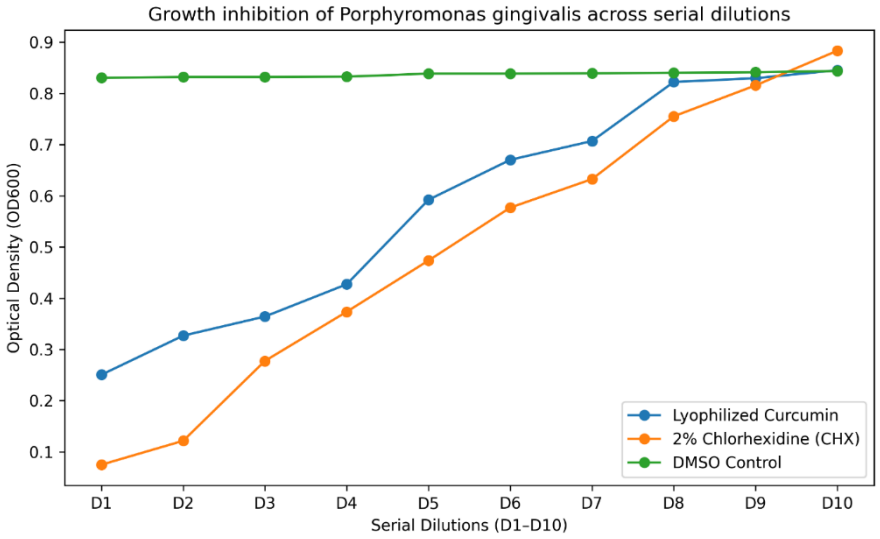

Figure 1. Abbreviations: CHX = chlorhexidine gluconate; DMSO = dimethyl sulfoxide.

Figure Legends

Figure 1. Growth inhibition of Porphyromonas gingivalis by lyophilized curcumin, chlorhexidine gluconate (CHX), and DMSO across serial dilutions (D1–D10) determined using a broth microdilution assay. Optical density (OD600) values indicate bacterial growth inhibition, with lower values representing greater antimicrobial activity.

Limitation of the study

Despite providing useful insights the present in-vitro study has certain limitations that should be considered when interpreting the findings. Specifically, the experiment was conducted under controlled laboratory conditions that do not fully replicate the complex biological environment of the periodontal pocket, where factors such as saliva, gingival crevicular fluid, host immune responses, and dynamic microbial interactions influence treatment outcomes. In addition, the antimicrobial activity was evaluated only against Porphyromonas gingivalis, whereas periodontitis is a polymicrobial disease involving complex multispecies biofilms; therefore, the results may not reflect the behaviour of mixed microbial communities.

The study focused primarily on bacterial growth inhibition and did not assess other clinically relevant parameters such as biofilm disruption, modulation of virulence factors, cytotoxicity to periodontal tissues, or host-modulatory effects. Furthermore, although lyophilized curcumin demonstrated antibacterial activity, its effectiveness may have been influenced by physicochemical limitations, including poor aqueous solubility and reduced bioavailability, and advanced formulations designed to enhance curcumin delivery were not investigated. The study also did not evaluate the substantivity or time-dependent antimicrobial effects of the tested agents, which are important considerations in periodontal therapy. Finally, because the investigation was conducted in vitro, the findings cannot be directly extrapolated to clinical situations, highlighting the need for in-vivo studies and well-designed clinical trials to confirm the therapeutic potential of curcumin in periodontal management.

DISCUSSION

This in-vitro study compared the antimicrobial efficacy of lyophilized curcumin and chlorhexidine gluconate against Porphyromonas gingivalis.

Chlorhexidine gluconate demonstrated superior antimicrobial activity, achieving approximately 90% bacterial inhibition at higher dilutions (D8). Lyophilized curcumin exhibited a significant inhibitory effect, achieving approximately 70% growth inhibition at D7. Curcumin offers anti-inflammatory and antioxidant effects, which may contribute to periodontal healing. The comparatively reduced antimicrobial efficacy of curcumin may be attributed to formulation-related limitations such as poor solubility and bioavailability. Within the limitations of this in-vitro study, chlorhexidine gluconate remains superior in antimicrobial potency. However, lyophilized curcumin demonstrates promising adjunctive potential. Further in-vivo studies focusing on optimized formulations, dosage standardization, and long-term clinical outcomes are recommended to better establish its role in periodontal therapy.

Declaration of Generative AI and AI-Assisted Technologies in Manuscript Preparation

In preparing this manuscript, the authors used AI tools to improve language clarity and manuscript organization. The authors reviewed and edited the content generated by these tools and take full responsibility for the final manuscript.

Ethical clearance

The study was in vitro and did not require ethical clearance certification

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Conflict of Interest

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data availability

Data generated and analyzed during this study are available from the corresponding author upon reasonable request.

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