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Terminalia chebula; Terminalia chebula seed extract (TCSE); Peri-implantitis; Biogel; Antibiofilm activity; Cytocompatibility.

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Terminalia chebula Seed Extract–Loaded Biogel as a Potential Alternative to Chlorhexidine for Early Peri-Implantitis: An In Vitro Evaluation

Abstract

Introduction: Peri-implantitis is a biofilm-mediated inflammatory disease that compromises implant longevity through persistent microbial colonization, oxidative stress, and progressive peri-implant bone loss. Although chlorhexidine (CHX) remains the clinical gold standard, its prolonged use is associated with cytotoxicity and undesirable adverse effects. Therefore, naturally derived biomaterials with antimicrobial, antibiofilm, antioxidant, and biocompatible properties are being explored as alternative therapeutic agents.

Aim: To formulate a Terminalia chebula seed extract (TCSE)-loaded biogel and evaluate its physicochemical characteristics, antimicrobial, antibiofilm, antioxidant, and cytocompatibility properties for the management of early peri-implantitis.

Materials and Methods: Ethanolic TCSE was incorporated into a gelatin–alginate biogel and characterized for pH, spreadability, viscosity, and swelling behavior. Phytochemical screening and DPPH assays were performed to determine bioactive constituents and antioxidant potential. Antimicrobial efficacy against Staphylococcus aureus and Porphyromonas gingivalis was assessed using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Antibiofilm activity was evaluated by crystal violet assay and confocal laser scanning microscopy. Cytocompatibility was determined using the MTT assay on MG-63 osteoblast-like cells.

Results: TCSE demonstrated abundant phenolics, flavonoids, tannins, terpenoids, and glycosides with concentration-dependent antioxidant activity. The optimized biogel exhibited near-neutral pH, desirable viscosity, adequate spreadability, and satisfactory swelling behavior. TCSE inhibited S. aureus (MIC/MBC: 250/500 µg/ml) and P. gingivalis (250/1000 µg/ml), with greater activity against P. gingivalis. The biogel significantly reduced biofilm biomass and bacterial viability in a concentration-dependent manner, confirmed by crystal violet assay and confocal microscopy. Although CHX exhibited superior antibacterial efficacy, TCSE-loaded biogel maintained excellent osteoblast viability across all tested concentrations, whereas CHX showed marked cytotoxicity.

Conclusion: TCSE-loaded biogel demonstrated promising multifunctional properties, combining antimicrobial, antibiofilm, antioxidant, and excellent cytocompatibility. These findings support its potential as a safe adjunctive local drug-delivery system for the management of early peri-implantitis.

INTRODUCTION

Peri-implantitis is a biofilm-mediated inflammatory disease characterized by progressive destruction of the peri-implant soft tissues and supporting alveolar bone, posing a significant threat to the long-term success of dental implants. With the increasing global use of implant-supported rehabilitation, the prevalence of peri-implant diseases has risen substantially, creating an urgent need for effective preventive and therapeutic strategies. The disease is initiated by the colonization of pathogenic microorganisms on titanium implant surfaces, where mature biofilms protect bacteria from host defences and antimicrobial agents, thereby limiting the effectiveness of conventional mechanical debridement and antiseptic therapy. Consequently, the development of adjunctive therapeutics capable of disrupting biofilms while preserving peri-implant tissue health has become a major research priority.

Beyond microbial infection, oxidative stress plays a pivotal role in peri-implant tissue destruction. Excessive reactive oxygen species generated during chronic inflammation amplify inflammatory signalling, stimulate osteoclast-mediated bone resorption, and impair tissue repair. Therefore, biomaterials that combine antimicrobial, antibiofilm, and antioxidant properties may provide a more comprehensive therapeutic approach than conventional antiseptics alone.

Natural phytochemicals have emerged as promising alternatives owing to their broad-spectrum antimicrobial activity, antioxidant potential, favourable biocompatibility, and lower risk of antimicrobial resistance. Terminalia chebula Retz., a renowned medicinal plant in traditional medicine, is particularly rich in hydrolysable tannins, flavonoids, phenolic acids, and other bioactive compounds with well-documented antimicrobial, antibiofilm, anti-inflammatory, antioxidant, and wound-healing activities. Incorporation of these phytoconstituents into a biogel delivery system offers the additional advantages of prolonged retention within the peri-implant sulcus, sustained local drug release, enhanced therapeutic efficacy, and minimal systemic exposure.

Despite the recognized pharmacological potential of T. chebula, evidence regarding its application as a localized biogel for peri-implant therapy remains limited. Therefore, this in vitro study aimed to formulate a Terminalia chebula seed extract-loaded biogel and comprehensively evaluate its antimicrobial, antibiofilm, and antioxidant efficacy, thereby exploring its potential as a multifunctional adjunct for the management of early peri-implantitis.

MATERIALS AND METHODS

2.1 Preparation of Terminalia chebula seed extracts (TCSE)

The ethanolic extract of Terminalia chebula seed was prepared by conventional maceration, a method selected to maximize the recovery of phenolic constituents while minimizing thermal degradation(1). Briefly, 50 g of dried seed powder was suspended in 500 ml of ethanol and maintained under continuous agitation (100 rpm) at room temperature (25°C) for five days. The extract was filtered through Whatman No. 1 filter paper, and the solvent was removed under reduced temperature (40°C) using a water bath to obtain a semi-solid crude extract(2). The concentrated extract was stored at −20°C until further use. Ethanol was selected as the extraction solvent because of its established efficiency in recovering polyphenols and flavonoids responsible for the antioxidant and antimicrobial activities of pomegranate seed extracts(3).

2.2 Phytochemical Characterization of Terminalia chebula Seed Extract (TCSE)

Preliminary phytochemical screening of the ethanolic Terminalia chebula seed extract (TCSE) was performed using standard qualitative pharmacognostic methods to identify the major classes of bioactive secondary metabolites. The extract was screened for alkaloids, carbohydrates, proteins, saponins, flavonoids, tannins, phenolic compounds, glycosides, terpenoids, steroids, and reducing sugars following established protocols described by Harborne (1998) and Evans (2009). Each phytochemical test was carried out in triplicate to ensure reproducibility. The presence or absence of individual phytochemical groups was determined based on the characteristic colour changes or precipitate formation observed during each qualitative assay(4).

2.3 Determination of Antioxidant Activity

The antioxidant capacity of TCSE was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Appropriate concentrations of the extract were mixed with DPPH solution and incubated in the dark at 37°C for 30 min. Absorbance was recorded at 517 nm using a UV–Visible spectrophotometer, with ascorbic acid serving as the reference antioxidant. Radical scavenging activity was expressed as percentage inhibition and calculated using established equations (Blois, 1958; Brand-Williams et al., 1995). The DPPH assay provides an estimate of the hydrogen atom-donating capacity of the extract and is widely accepted as an indicator of antioxidant potential relevant to oxidative stress-associated tissue injury(5).

2.4 Preparation of TCSE -Loaded Biogel

The hydrogel matrix was prepared using gelatin and sodium alginate because of their established biocompatibility, biodegradability, and suitability for localized drug delivery applications(6). Gelatin was dissolved in distilled water at 40–50°C, whereas sodium alginate was prepared separately at room temperature. The polymer solutions were combined under continuous stirring, followed by incorporation of Polyox to improve viscosity and handling characteristics. Ionic crosslinking was achieved through gradual addition of calcium chloride, producing a stable three-dimensional polymeric network(7). TCSE was subsequently dispersed uniformly throughout the gel matrix to obtain the final formulation. The gelatin–alginate system was selected because its hydrated structure facilitates sustained release of encapsulated bioactive compounds while providing a

microenvironment that resembles the extracellular matrix(8).

2.5 Physicochemical Characterization

2.5.1 pH Determination

The pH of the optimized formulation was measured using a calibrated digital pH meter to confirm compatibility with the physiological conditions of the oral cavity(9).

2.5.2 Spreadability and Rheological Evaluation

Spreadability was determined by the parallel-plate method, while viscosity measurements were performed using a Brookfield viscometer across a range of shear rates. These parameters were evaluated to determine the ease of clinical application, retention at the implant site, and overall handling characteristics of the formulation(10).

2.5.3 Swelling Behaviour

Hydrogel swelling was evaluated gravimetrically by immersing pre-weighed samples in distilled water and calculating the percentage increase in mass. Swelling behaviour provides insight into fluid uptake, bioadhesion, and the potential release characteristics of encapsulated bioactive compounds(11).

Antimicrobial Activity

Minimum Inhibitory Concentration (MIC)

The antimicrobial activity of the TCSE -loaded biogel was evaluated against Porphyromonas gingivalis (ATCC 33277) and Staphylococcus aureus (ATCC 25923) using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

P. gingivalis was cultured on blood agar supplemented with 1% horse serum, hemin, and vitamin K under anaerobic conditions (N_{222}; 80:10:10) using an anaerobic workstation (KIM Microsystems, India). Colonies were suspended in fresh brain heart infusion (BHI) broth and adjusted to a turbidity equivalent to a 0.5 McFarland standard. S. aureus susceptibility testing was performed under aerobic conditions. Serial dilutions of the formulation were prepared in BHI broth, and MIC values were recorded as the lowest concentration preventing visible bacterial growth after incubation (CLSI, 2020)(12).

2.5.4 Minimum Bactericidal Concentration (MBC)

Aliquots from wells showing no visible growth in the MIC assay were subcultured onto agar plates to determine bactericidal activity. The MBC was defined as the lowest concentration producing complete bacterial killing, as evidenced by the absence of colony formation(13).

2.5.5 Antibiofilm Activity

Biofilm inhibition was assessed using a crystal violet staining assay. Biofilms were established in BHI broth under anaerobic conditions for 72 h before treatment with TCSE -loaded biogel at concentrations corresponding to 1xMIC and 2xMIC for an additional 24 h. Following methanol fixation, biofilms were stained with 0.1% crystal violet, washed with phosphate-buffered saline, and destained using 95% ethanol. Optical density was measured at 570 nm using a microplate reader, providing a quantitative estimate of residual biofilm biomass (14).

RESULTS

3.1 Preparation of Terminalia chebula seed extracts (TCSE)

Figure 1. Ethanolic extract of Terminalia chebula seed (TCSE) obtained after solvent extraction.
The ethanolic extract of Terminalia chebula seed (TCSE) was successfully obtained following solvent extraction. The extract appeared as a dark brown to black, semi-solid crude mass with a characteristic herbal odour, indicating efficient recovery of phytoconstituents from the seeds as shown in the (Figure 1).

3.2 Phytochemical Characterization of Terminalia chebula Seed Extract

Qualitative phytochemical analysis demonstrated that the ethanolic Terminalia chebula seed extract contained a diverse spectrum of bioactive secondary metabolites (Table 1). Alkaloids, carbohydrates, saponins, flavonoids, tannins, phenolic compounds, glycosides, terpenoids, steroids, and reducing sugars were detected in the extract, whereas proteins were absent.

The abundant occurrence of phenolic compounds and hydrolysable tannins is consistent with the well-established phytochemical profile of Terminalia chebula. These constituents are considered the principal contributors to the extract's biological activity owing to their pronounced antioxidant capacity and their ability to modulate microbial growth and inflammatory responses. The coexistence of flavonoids, alkaloids, and saponins further indicates that multiple phytochemical classes may act synergistically to produce the pharmacological effects associated with the extract.

Table 1: Phytochemical Characterization of Terminalia chebula Seed Extract

SL. No.	Phytochemical	TCSE
1	Alkaloids	+ Present
2	Proteins	− Absent
3	Carbohydrates	+ Present
4	Saponins	+ Present
5	Flavonoids	+ Present
6	Phenolic compounds	+ Present
7	Tannins	+ Present
8	Glycosides	+ Present
9	Terpenoids	+ Present
10	Steroids	+ Present
11	Reducing sugars	+ Present

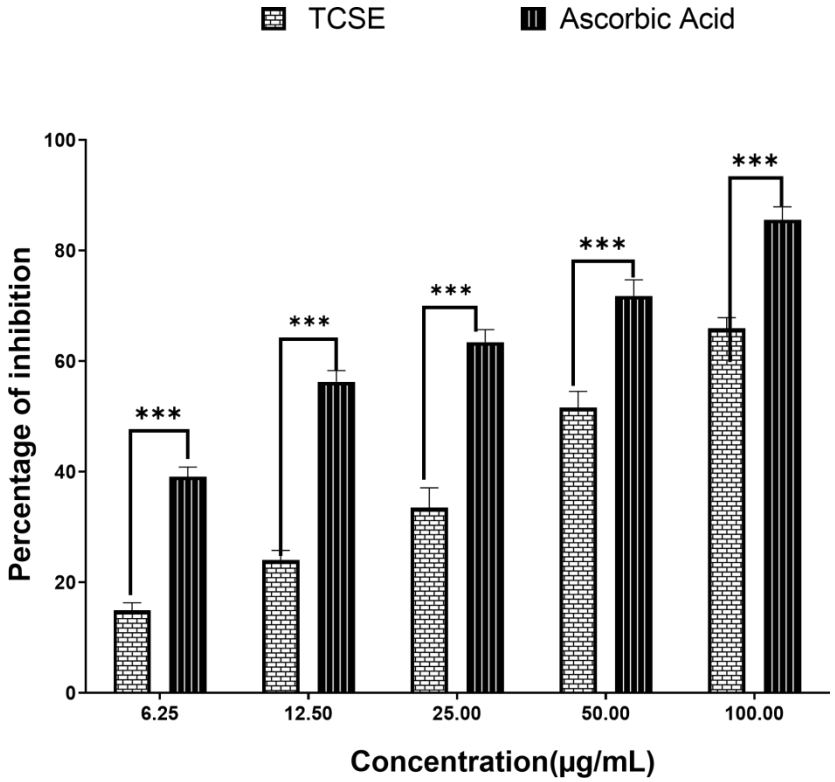

Figure 2. DPPH free radical scavenging activity of Terminalia chebula seed extract (TCSE) compared with ascorbic acid.

The DPPH free radical scavenging assay demonstrated a concentration-dependent increase in the antioxidant activity of both TCSE and the reference standard, ascorbic acid (Figure 2). TCSE exhibited progressive

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radical scavenging activity, increasing from approximately 15% inhibition at 6.25 µg/ml to 24%, 33%, 51%, and 66% inhibition at 12.5, 25, 50, and 100 µg/ml, respectively. In comparison, ascorbic acid showed significantly higher antioxidant activity at all tested concentrations, with percentage inhibition of approximately 39%, 56%, 63%, 72%, and 85%, respectively.

At each concentration, the antioxidant activity of ascorbic acid was significantly greater than that of TCSE (**P < 0.001). Despite being less potent than the standard antioxidant, TCSE demonstrated a marked dose-dependent increase in free radical scavenging capacity, achieving approximately two-thirds inhibition at the highest tested concentration (100 µg/ml).

Figure 3. TCSE-loaded biogel formulated using Terminalia chebula seed extract.

The TCSE-loaded biogel was successfully formulated as a homogeneous, translucent pale-yellow gel with a smooth and uniform consistency. The formulation showed no evidence of phase separation, precipitation, or entrapped air bubbles, indicating excellent physical stability and uniform distribution of the Terminalia chebula seed extract within the gel matrix as shown in the (Figure 3).

3.3 Physicochemical Characterization

3.2.1 pH Determination

The blank hydrogel showed a mean pH of 6.83, whereas the TCSE-loaded hydrogel exhibited a mean pH of 6.07. Both formulations-maintained a near-neutral pH, indicating their suitability for oral application with minimal risk of mucosal irritation.

Table 3. pH of Blank and TCSE-Loaded Hydrogel

Formulation	Trial 1	Trial 2	Trial 3	Mean pH
Blank hydrogel	6.7	6.86	6.92	6.82666667
Extract-loaded hydrogel	6.1	6.22	5.89	6.07

3.2.2 Spreadability and Rheological Evaluation

The blank hydrogel demonstrated a mean spreadability of 5.37 cm, while the TCSE-loaded hydrogel showed a mean spreadability of 4.93 cm. Incorporation of TCSE resulted in a slight reduction in spreadability; however, the formulation retained satisfactory consistency for topical intraoral application.

Table 4. Spreadability of Blank and TCSE-Loaded Hydrogel

Formulation	Trial 1 (cm)	Trial 2 (cm)	Trial 3 (cm)	Mean (cm)
Blank hydrogel	5.4	5.2	5.5	5.37
TCSE-loaded hydrogel	5	4.7	5.1	4.93

The TCSE-loaded hydrogel exhibited higher viscosity than the blank hydrogel across the tested rotational speeds. At 0.5 rpm, the viscosity increased from 27,532 cP (blank gel) to 32,200 cP (TCSE-loaded gel), indicating improved structural integrity following extract incorporation. Both formulations demonstrated shear-dependent viscosity, a desirable characteristic for topical gels that facilitates ease of application while maintaining retention at the target site.

Table 6. MIC and MBC of TCSE Against Test Organisms

RPM	Viscosity Blank Gel (Cp)	Viscosity of TCSE (Cp)
0.5	27,532	32,200
1	14,522	14.7
2	4,567	5600
5	7152	8200

3.2.3 Swelling Behaviour

The TCSE-loaded hydrogel exhibited a mean swelling index of 48.50 ± 5.23%, indicating good water uptake capacity. The gel also demonstrated a mean weight gain of 10.67 ± 0.68 g following hydration. These findings suggest adequate swelling behavior, which is desirable for sustained drug release and prolonged retention at the application site.

The antimicrobial evaluation demonstrated that TCSE exhibited inhibitory activity against both Staphylococcus aureus and Porphyromonas gingivalis. For S. aureus, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were 250µg/mL and 500 µg/mL, respectively. Against P. gingivalis, TCSE showed greater antibacterial potency with MIC and MBC values of 250 µg/mL and 1000 µg/mL, respectively. Chlorhexidine exhibited substantially lower MIC and MBC values for both organisms, confirming its superior antimicrobial efficacy; however, TCSE demonstrated appreciable antibacterial activity, particularly against P. gingivalis, supporting its potential as a natural antimicrobial agent for peri-implant applications.

Table 6. MIC and MBC of TCSE Against Test Organisms

		Staphylococcus Aureus		P. Gingivalis	
SLNO	Sample Codes	MIC	MBC	MIC	MBC
1	TCSE	250	500	250	1000
2	CHX	62.5	125	31.25	62.5

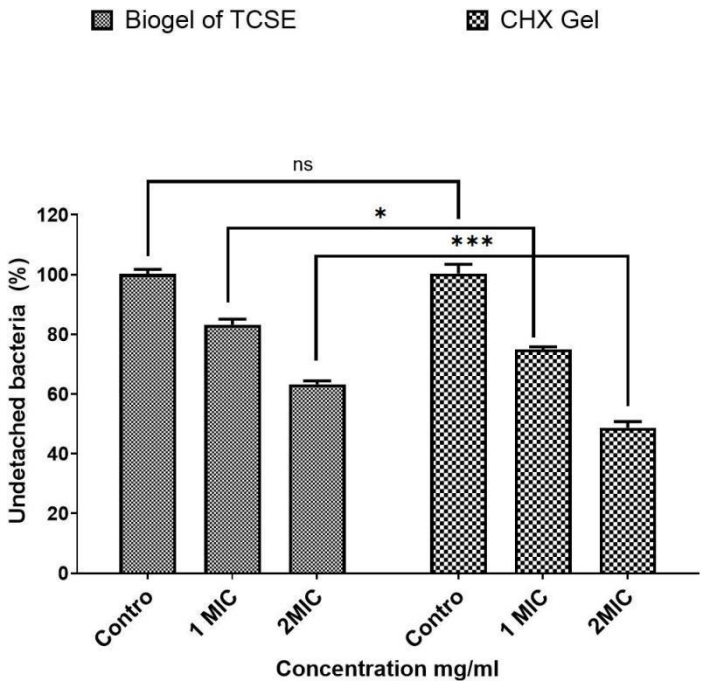

Figure 4. Antibiofilm efficacy of Terminalia chebula seed extract (TCSE)-loaded biogel and chlorhexidine (CHX) gel against Staphylococcus aureus biofilm, expressed as the percentage of undetached bacteria.

The antibiofilm assay demonstrated that both TCSE-loaded biogel and chlorhexidine (CHX) gel significantly reduced the proportion of undetached Staphylococcus aureus biofilm in a concentration-dependent manner (Figure 4). The untreated control groups for both TCSE-loaded biogel and CHX gel exhibited approximately 100% undetached bacteria, with no significant difference between them (ns, $P > 0.05$).

Treatment with TCSE-loaded biogel reduced the percentage of undetached bacteria from 100% in the control to approximately 83% at 1xMIC and 63% at 2xMIC, demonstrating a significant concentration-dependent antibiofilm effect. Compared with the untreated control, the reduction observed at 1xMIC was statistically significant ($P < 0.05$), while 2xMIC produced a more pronounced decrease in biofilm biomass.

Similarly, CHX gel exhibited superior antibiofilm activity, decreasing the percentage of undetached bacteria to approximately 75% at 1xMIC and 48% at 2xMIC. The reduction at 2xMIC was highly significant compared with the control (*** $P < 0.001$). At equivalent

concentrations, CHX gel produced greater biofilm disruption than TCSE-loaded biogel, particularly at 2xMIC, indicating stronger biofilm detachment.

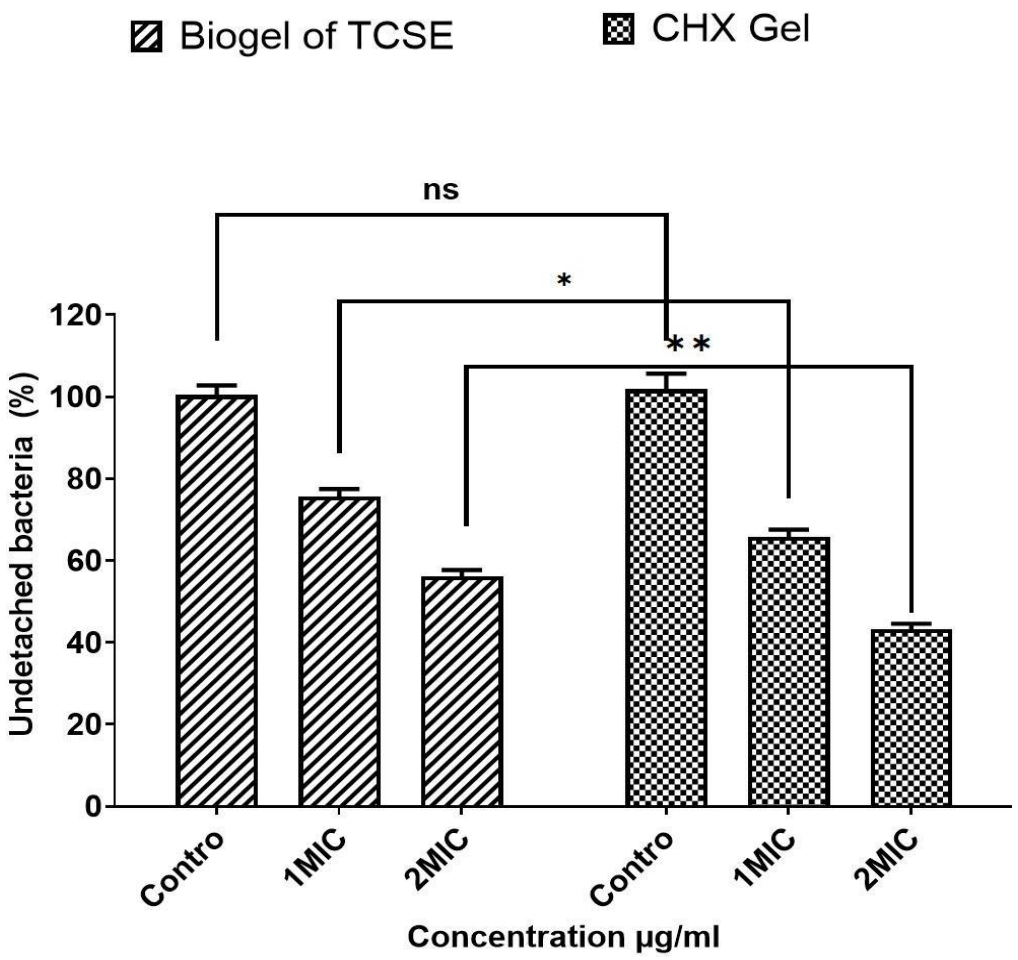

Figure 5. Antibiofilm activity of Terminalia chebula seed extract (TCSE)-loaded biogel and chlorhexidine (CHX) gel against established Staphylococcus aureus biofilm, expressed as the percentage of undetached bacteria.

The antibiofilm assay demonstrated that both TCSE-loaded biogel and chlorhexidine (CHX) gel effectively disrupted established Staphylococcus aureus biofilms in a concentration-dependent manner (Figure 5). The untreated control groups showed approximately 100% undetached bacteria, with no significant difference between the TCSE-loaded biogel and CHX gel groups (ns, $P > 0.05$).

Treatment with TCSE-loaded biogel significantly reduced the proportion of undetached bacteria from 100% in the control to approximately 75% at 1xMIC and 56% at 2xMIC, indicating enhanced biofilm detachment with increasing concentration. Similarly, CHX gel reduced the percentage of undetached bacteria to approximately 65% at 1xMIC and 43% at 2xMIC.

Comparison between the two formulations revealed that CHX gel exhibited significantly greater antibiofilm activity than TCSE-loaded biogel at corresponding concentrations. At 1xMIC, CHX gel produced a significantly greater reduction in undetached bacteria than TCSE-loaded biogel ($P < 0.05$), while at 2xMIC, the difference remained significant (*$P < 0.01$). Despite the superior biofilm-disrupting effect of CHX gel, TCSE-loaded biogel demonstrated substantial concentration-dependent antibiofilm activity, achieving nearly a 44% reduction in undetached bacteria at 2xMIC. These findings suggest that TCSE-loaded biogel possesses promising natural antibiofilm properties against S. aureus and may serve as a biocompatible adjunctive therapeutic agent for the management of early peri-implantitis.

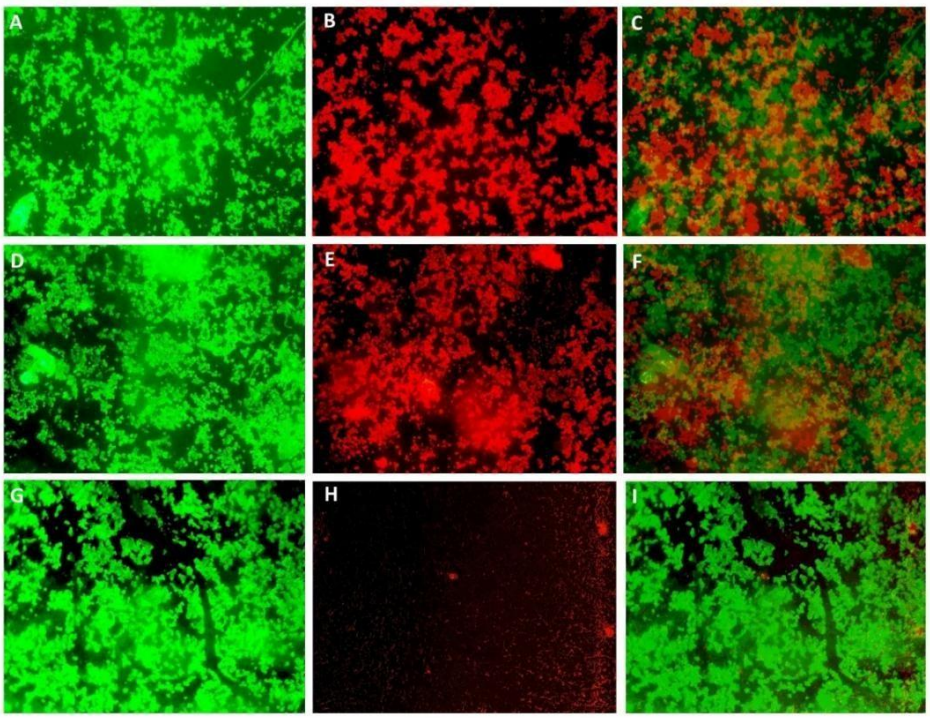

Figure 6. Confocal laser scanning microscopy (CLSM) images of Staphylococcus aureus biofilms stained with acridine orange (AO; green, total/live bacterial cells) and ethidium bromide (EtBr; red, membrane-compromised/dead bacterial cells) following treatment with TCSE-loaded biogel. (A–C) Biofilms treated with TCSE biogel at 1x MIC: (A) AO staining, (B) EtBr staining, and (C) merged image. (D–F) Biofilms treated with TCSE biogel at 2x MIC: (D) AO staining, (E) EtBr staining, and (F) merged image. (G–I) Untreated control biofilms: (G) AO staining, (H) EtBr staining, and (I) merged image. Increased red fluorescence and reduced green fluorescence in the treated groups indicate enhanced bacterial cell death and disruption of the S. aureus biofilm, with the 2x MIC group exhibiting greater antibiofilm activity than the 1x MIC group.

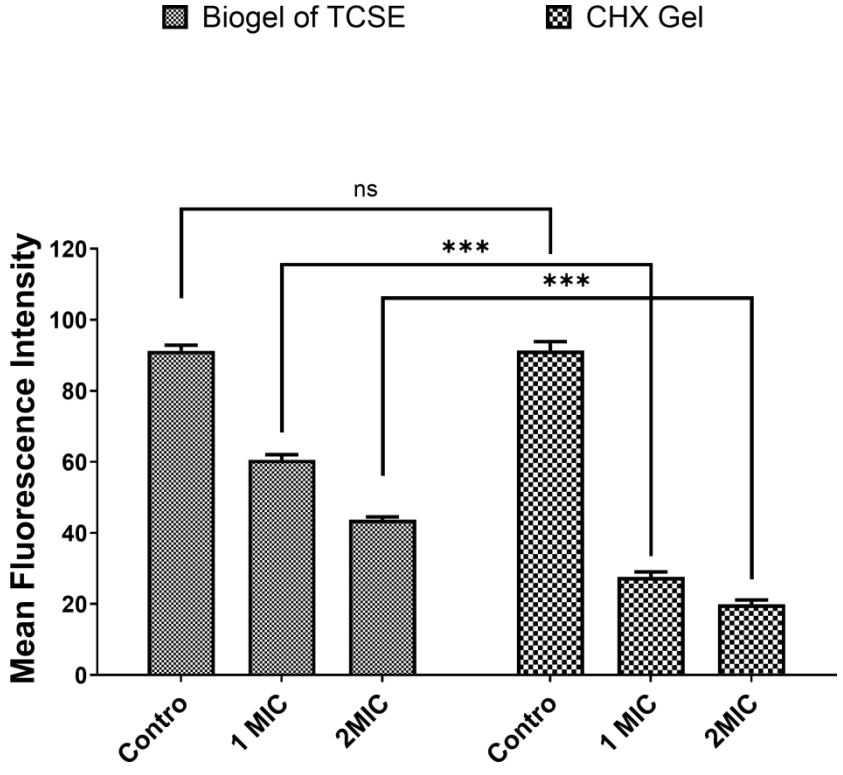

Figure 7. The anti-biofilm activity of Terminalia chebula seed extract (TCSE) biogel was compared with the chlorhexidine (CHX) gel by measuring the mean fluorescence intensity of live bacterial cells. As shown in the figure, the untreated control groups of both TCSE biogel and CHX gel exhibited high fluorescence intensity

(approximately 90 arbitrary units), indicating a high proportion of viable bacteria. There was no significant difference between the untreated control groups (ns, p > 0.05).

Treatment with both formulations at 1× MIC significantly reduced bacterial fluorescence compared with their respective untreated controls (*p < 0.001). The TCSE biogel reduced the mean fluorescence intensity from approximately 90 to 60, whereas the CHX gel produced a greater reduction to approximately 27. At 2× MIC, fluorescence intensity decreased further, reaching approximately 43 for the TCSE biogel and 20 for the CHX gel (*p < 0.001 versus control).

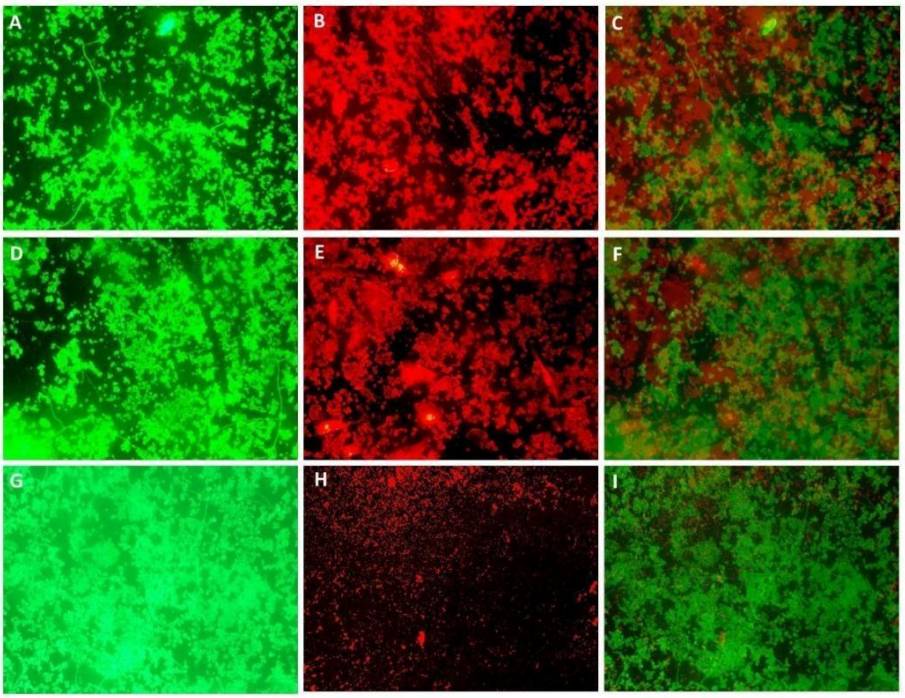

Figure 8. Confocal laser scanning microscopy (CLSM) images of P. gingivalis biofilms stained with acridine orange (AO; green, total/live bacterial cells) and ethidium bromide (EtBr; red, membrane-compromised/dead bacterial cells) following treatment with TCSE-loaded biogel. (A–C) Biofilms treated with TCSE biogel at 1x MIC: (A) AO staining, (B) EtBr staining, and (C) merged image. (D–F) Biofilms treated with TCSE biogel at 2x MIC: (D) AO staining, (E) EtBr staining, and (F) merged image. (G–I) Untreated control biofilms: (G) AO staining, (H) EtBr staining, and (I) merged image. Increased red fluorescence and reduced green fluorescence in the treated groups indicate enhanced bacterial cell death and disruption of the S. aureus biofilm, with the 2x MIC group exhibiting greater antibiofilm activity than the 1x MIC group.

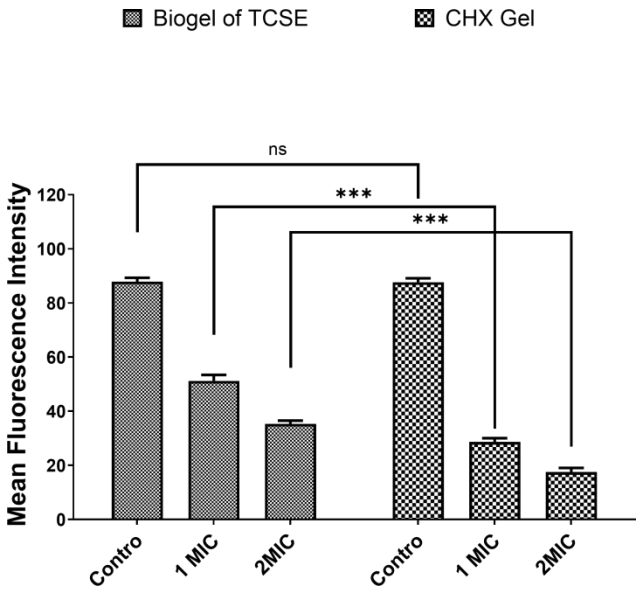

Figure 9. The anti-biofilm efficacy of the Terminalia chebula seed extract (TCSE) biogel and the chlorhexidine (CHX) gel was assessed by measuring the mean fluorescence intensity of bacterial cells following treatment at 1× MIC and 2× MIC (Figure X). The untreated control groups of both TCSE biogel and CHX gel exhibited

comparable fluorescence intensities (approximately 87–88 arbitrary units), with no significant difference between them ($p > 0.05$).

Treatment with the TCSE biogel significantly reduced bacterial fluorescence intensity from 87.5 ± 1.2 in the control group to 50.6 ± 2.1 at 1× MIC and further to 35.0 ± 1.5 at 2× MIC ($p < 0.001$ vs. control), indicating a concentration-dependent reduction in bacterial viability. Similarly, the CHX gel produced a marked decrease in fluorescence intensity from 87.3 ± 1.4 in the control group to 28.2 ± 1.3 at 1× MIC and 17.1 ± 1.2 at 2× MIC ($p < 0.001$ vs. control). The reduction observed with CHX gel was significantly greater than that achieved with the TCSE biogel at the corresponding MIC concentrations, demonstrating superior antibacterial activity.

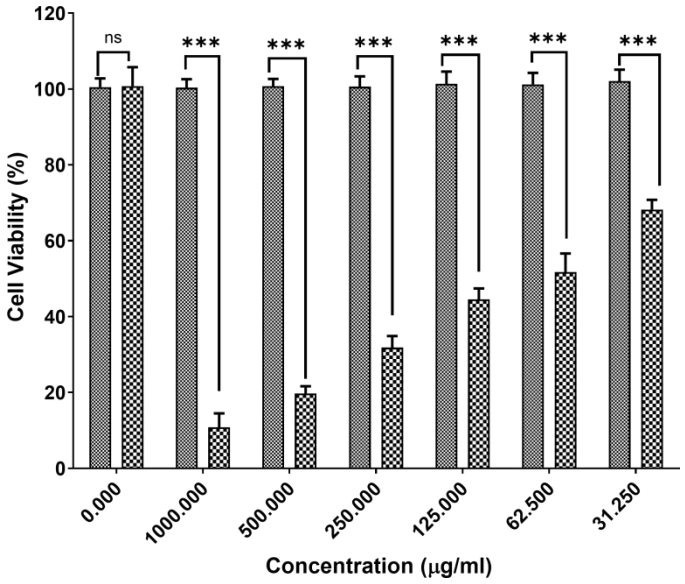

Figure 10. Cytocompatibility of Terminalia chebula seed extract (TCSE)-loaded biogel and chlorhexidine (CHX) gel on MG-63 osteoblast-like cells assessed by the MTT assay after 24 h of exposure.

The MTT assay demonstrated a marked difference in the cytocompatibility of TCSE-loaded biogel and chlorhexidine (CHX) gel toward MG-63 osteoblast-like cells (Figure 10). The untreated control groups exhibited approximately 100% cell viability, with no significant difference between the two groups ($P > 0.05$). TCSE-loaded biogel maintained excellent cytocompatibility throughout the tested concentration range (31.25–1000 µg/ml), with cell viability remaining close to 100%, indicating negligible cytotoxic effects even at the highest concentration.

In contrast, CHX gel exhibited a pronounced concentration-dependent cytotoxic effect. Cell viability was reduced to approximately 10% at 1000 µg/ml, increasing gradually to 20%, 31%, 45%, 52%, and 68% at 500, 250, 125, 62.5, and 31.25 µg/ml, respectively. At every tested concentration, the viability of MG-63 cells treated with TCSE-loaded biogel was significantly higher than that of cells treated with CHX gel (*** $P < 0.001$).

DISCUSSION

The present study demonstrated that Terminalia chebula seed extract (TCSE)-loaded biogel possesses a unique combination of antimicrobial, antibiofilm, antioxidant, and physicochemical properties that are highly desirable for the management of early peri-implantitis. Although chlorhexidine (CHX) exhibited superior antibacterial potency, TCSE-loaded biogel showed significant biofilm inhibition with the additional advantage of excellent cytocompatibility, highlighting its potential as a biologically favourable alternative for localized peri-implant therapy(15). These findings support the growing paradigm that successful peri-implantitis management should simultaneously target microbial biofilms, oxidative stress, and preservation of host tissue rather than relying solely on antimicrobial activity.

Phytochemical screening confirmed the presence of hydrolysable tannins, phenolic compounds, flavonoids, alkaloids, terpenoids, glycosides, and saponins, which are recognized contributors to the biological activity of T. chebula(16). Hydrolysable tannins, particularly chebulagic acid and chebulinic acid, have been shown to disrupt bacterial cell membranes, inhibit extracellular polysaccharide synthesis, interfere with quorum sensing, and suppress biofilm maturation. Likewise, flavonoids and phenolic acids exhibit broad-spectrum antimicrobial effects through membrane destabilization and inhibition of essential bacterial enzymes(17). The synergistic interaction among these phytoconstituents likely explains the concentration-dependent antibacterial and antibiofilm activities observed in the present study. Previous phytochemical investigations by Saleem et al. and Bag et al. similarly attributed the medicinal efficacy of T. chebula to its rich polyphenolic composition, particularly its high tannin content(18).

One of the most clinically relevant findings was the potent antioxidant activity demonstrated by TCSE in the DPPH assay. Although its radical scavenging capacity was lower than that of ascorbic acid, the extract

exhibited a significant dose-dependent increase in antioxidant activity. Oxidative stress plays a central role in peri-implantitis by amplifying inflammatory cytokine release, stimulating osteoclastogenesis through the RANKL pathway, and impairing osteoblastic function(19). Therefore, antioxidants capable of neutralizing reactive oxygen species may interrupt the destructive inflammatory cascade and facilitate peri-implant tissue healing. Unlike CHX, which primarily functions as an antiseptic, TCSE provides simultaneous antioxidant protection, making it a multifunctional therapeutic candidate.

The formulated TCSE biogel exhibited physicochemical characteristics suitable for local drug delivery. The near-neutral pH, adequate spreadability, increased viscosity, and favourable swelling behaviour indicate that the formulation can remain within the peri-implant sulcus for prolonged periods while allowing sustained release of bioactive compounds(20). Gelatin–alginate hydrogels closely resemble the extracellular matrix and have been extensively reported to enhance drug retention and controlled release at oral wound sites. Such localized delivery may overcome one of the major limitations of conventional mouth rinses by maintaining prolonged therapeutic concentrations directly at infected implant surfaces(21).

The antimicrobial evaluation demonstrated that TCSE inhibited both Staphylococcus aureus and Porphyromonas gingivalis, with greater activity against P. gingivalis. The enhanced susceptibility of P. gingivalis may be related to differences in cell wall composition and increased sensitivity of anaerobic pathogens to plant-derived polyphenols(22). Although CHX exhibited lower MIC and MBC values, indicating greater bactericidal potency, complete bacterial eradication alone may not represent the ideal therapeutic endpoint in peri-implantitis because excessive antimicrobial exposure can adversely affect host cells and disturb the oral microbiome(23).

Biofilm disruption is considerably more challenging than inhibition of planktonic bacteria because the extracellular polymeric matrix limits antimicrobial penetration(24). In the present investigation, TCSE-loaded biogel significantly reduced biofilm biomass and bacterial viability in a concentration-dependent manner, as demonstrated by crystal violet assays and confocal laser scanning microscopy. CLSM images revealed increased red fluorescence and diminished green fluorescence following treatment, confirming membrane damage and bacterial death within established biofilms(25). These observations suggest that TCSE interferes not only with bacterial growth but also with biofilm architecture and structural integrity. Nevertheless, CHX remained more effective in disrupting mature biofilms, consistent with its well-established membrane-disruptive mechanism(26). Importantly, the substantial antibiofilm activity demonstrated by TCSE indicates that natural phytochemicals may provide clinically meaningful adjunctive effects while avoiding many of the adverse effects associated with prolonged CHX use, including mucosal irritation, tooth staining, dysgeusia, and cytotoxicity(27).

Perhaps the most significant observation of this study was the remarkable cytocompatibility of the TCSE-loaded biogel. MG-63 osteoblast-like cells maintained nearly 100% viability across all tested concentrations, whereas CHX produced severe concentration-dependent cytotoxicity. Preservation of osteoblast viability is particularly important because successful peri-implant therapy requires not only elimination of infection but also regeneration of peri-implant bone(28). Previous investigations have consistently shown that CHX, even at clinically relevant concentrations, adversely affects fibroblasts, osteoblasts, and stem cells by inducing oxidative stress, mitochondrial dysfunction, and apoptosis(29). In contrast, polyphenolic compounds present in T. chebula possess antioxidant and anti-inflammatory properties that protect host cells while suppressing microbial colonization, thereby offering a superior biological profile for regenerative peri-implant therapy(30).

The present study has certain limitations. Being an in vitro investigation, it does not fully replicate the complex oral environment, where salivary proteins, multispecies biofilms, mechanical loading, and host immune responses influence treatment outcomes. Furthermore, only two bacterial species were evaluated, whereas peri-implantitis involves a polymicrobial biofilm. Future studies should investigate multispecies biofilms, release kinetics, long-term stability, animal models, and randomized clinical trials to validate the translational potential of TCSE-loaded biogel.

CONCLUSION

The present in vitro study demonstrated that the Terminalia chebula seed extract (TCSE)-loaded biogel possesses promising antimicrobial, antibiofilm, and antioxidant properties while maintaining excellent physicochemical characteristics and cytocompatibility. Although chlorhexidine exhibited superior antibacterial efficacy, TCSE-loaded biogel effectively inhibited both Staphylococcus aureus and Porphyromonas gingivalis, disrupted established biofilms in a concentration-dependent manner, and preserved osteoblast viability. The synergistic action of its phytoconstituents, together with sustained local delivery through the biogel matrix, suggests that TCSE-loaded biogel represents a safe and multifunctional therapeutic approach for the management of early peri-implantitis. However, further in vivo studies and well-designed clinical trials are required to validate its long-term clinical efficacy and safety before routine clinical application.

CLINICAL IMPLICATIONS

TCSE-loaded biogel has the potential to serve as a natural, biocompatible adjunct to conventional non-surgical peri-implant therapy. Its ability to simultaneously reduce microbial biofilms, neutralize oxidative stress, and preserve osteoblast viability may promote a more favorable peri-implant healing environment than conventional antiseptics alone. The localized gel formulation offers prolonged retention within the peri-implant sulcus and sustained release of bioactive compounds, making it a promising alternative to chlorhexidine, particularly for long-term management

where minimizing cytotoxicity and adverse effects is desirable. Future clinical studies should evaluate its effectiveness in reducing peri-implant inflammation, preventing disease progression, and supporting peri-implant tissue regeneration.

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