



Exploring the Stability, Molecular Integrity and Potent *Ex Vivo* Antimicrobial Efficacy of a Catechin-enriched Effervescent Denture Cleanser

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Abstract

Denture-associated stomatitis is primarily driven by persistent microbial biofilm accumulation on prosthetic surfaces. Bioactive phytochemicals such as catechins from *Camellia sinensis* possess documented antimicrobial and antioxidant properties; however, their translation into stable effervescent denture-cleansing formulations remains insufficiently characterized. This study aimed to assess the antimicrobial performance of UV-standardized aqueous *Camellia sinensis* extract on patient-derived dentures under *ex vivo* conditions and to develop and evaluate a catechin-enriched effervescent tablet by examining its physicochemical, spectroscopic, antimicrobial, cytocompatibility and structural stability characteristics. The formulated effervescent tablets showed consistent dimensional accuracy, hardness ($4.83 \pm 0.22 \text{ kg cm}^{-2}$), friability ($0.12 \pm 0.02\%$), rapid disintegration ($40 \pm 2 \text{ s}$), complete effervescence ($88 \pm 3.2 \text{ s}$) and mildly acidic pH (5.38 ± 0.10), consistent with effervescent formulation requirements. Spectroscopic analysis showed retention of the characteristic catechin functional groups, with no major spectral changes attributable to interactions with formulation excipients. The reconstituted solution showed a minimum inhibitory concentration of $0.9 \mu\text{g mL}^{-1}$ against both organisms and maintained $>90\%$ fibroblast viability ($\text{IC}_{50} = 467.3 \mu\text{g mL}^{-1}$). Sequential *ex vivo* and *in vitro* analyses showed that UV-standardized aqueous *Camellia sinensis* extract retained antimicrobial potency and can be incorporated into an effervescent tablet that is rapidly dispersible, structurally stable and biocompatible, with appreciable antimicrobial activity, supporting its suitability as a denture-cleansing formulation. Further investigations using advanced biofilm models and clinical conditions are warranted to validate long-term efficacy.

Keywords: Antimicrobial, *Camellia sinensis*, Catechin, Cytotoxicity, Denture cleanser, Effervescent Tablet.

Introduction

Biofilm accumulation on removable prostheses remains a persistent clinical challenge in prosthodontic practice. Polymethyl methacrylate (PMMA), the principal material used for denture bases, possesses surface micro-irregularities and hydrophilic-hydrophobic domains that facilitate the adhesion and maturation of mixed microbial communities (1). The formation of mixed-species biofilms involving *Candida albicans*, *Staphylococcus aureus* and other opportunistic organisms contributes to inflammatory changes collectively recognized as denture stomatitis (2). This condition manifests as erythema, burning sensation and malodor and, in susceptible

individuals, especially the elderly and immune-compromised, may predispose to secondary systemic dissemination of pathogens (3). These outcomes underscore the need for a biocompatible and functionally stable cleansing modality capable of achieving effective microbial decontamination without compromising PMMA surface properties. Conventional chemical denture cleansers, such as alkaline peroxides, Sodium hypochlorite and chlorhexidine-based formulations, are widely used owing to their immediate antimicrobial action and ease of application. Nevertheless, long-term use of these agents has been associated with surface micro-abrasion, reduction in flexural strength,

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alteration in color stability and potential cytotoxicity to oral tissues (4). Furthermore, chlorhexidine formulations often cause transient mucosal irritation and an unpleasant lingering taste sensation that affects patient compliance (5). These limitations have prompted research into safer, biologically derived alternatives that combine antimicrobial activity with favourable material compatibility and patient acceptability.

In recent years, several herbal and phytochemical agents have been investigated for their antimicrobial potential in denture hygiene applications. Extracts of *Azadirachta indica* (neem), *Aloe vera*, *Ocimum sanctum* (tulsi) and *Curcuma longa* (turmeric) have demonstrated varying degrees of antibacterial or antifungal activity in oral biofilm models (6-9). However, inconsistencies in bioactive standardization, poor solubility and instability under aqueous conditions have restricted their formulation into effective and reproducible cleansing systems. Among these botanicals, *Camellia sinensis* (green tea) has emerged as a promising candidate owing to its well-characterized polyphenolic composition, abundant catechin content and well-documented biological activities (10).

Green tea is particularly enriched with catechin-class polyphenols, with epigallocatechin gallate (EGCG) being the most bioactive constituent due to its potent antioxidant, antibacterial and antifungal properties. These activities are mediated through multiple mechanisms including disruption of microbial membrane integrity, inhibition of virulence-associated enzymes, chelation of essential metal ions and interference with quorum-sensing pathways that regulate biofilm maturation (11). EGCG also demonstrates the ability to suppress adhesion of *Candida albicans* to PMMA surfaces, suggesting potential as a denture cleansing agent that mitigates microbial colonization without chemical abrasion or material compromise (12). Despite these mechanistic advantages, there remains a lack of translational formulations that deliver standardized catechin concentrations in a stable, user-friendly and patient-compliant dosage form suitable for prosthodontic application.

However, current literature lacks comprehensive studies that bridge phytochemical standardization with formulation science in denture hygiene.

Previous works have primarily focused on solution-based or mouth rinse forms of green tea, with limited evidence supporting their long-term stability or patient usability. Accordingly, this study was designed to establish a catechin effervescent system capable of coupling phytochemical integrity with functional antimicrobial and biocompatibility performance, addressing a critical gap in prosthetic hygiene formulations.

Effervescent tablets represent an advanced pharmaceutical platform that ensures rapid disintegration, uniform dispersion and complete solubilization of the active ingredient upon contact with water. The effervescence generated through the acid-base interaction between citric acid and sodium bicarbonate enhances dissolution kinetics and facilitates homogeneous distribution of the bioactive compound (13). This delivery system offers dose uniformity, portability and improved patient acceptance, while maintaining a mildly acidic pH, thereby preventing potential alterations in PMMA surface characteristics. Incorporating standardized *Camellia sinensis* extract into such an effervescent matrix provides a rational strategy to transform catechin bioactivity into a stable, reproducible and user-friendly denture-cleansing modality.

The present study was undertaken to formulate and characterize a catechin-enriched *Camellia sinensis* effervescent tablet and to evaluate its antimicrobial and physicochemical performance through comprehensive *in vitro* analyses. Additionally, the standardized aqueous extract of *C. sinensis* was tested *ex vivo* on patient-derived dentures to assess its immediate microbial decontamination efficacy in comparison with distilled water and 0.2% chlorhexidine solution. This integrated approach combines phytochemical evaluation, formulation development and *ex vivo* assessment within a prosthodontic context.

The null hypothesis was that the antimicrobial efficacy of the *Camellia sinensis*-based interventions (effervescent formulation and standardized extract) does not differ significantly from that of the conventional control solutions when evaluated under *in vitro* and *ex vivo* conditions.

Methodology

Study Design and Ethical Approval

A prospectively structured study with controlled group allocation was designed with two complementary components comprising *in vitro* evaluation of the formulated catechin-enriched *Camellia sinensis* effervescent tablet and *ex vivo* assessment of the standardized green tea extract on patient-derived denture surfaces.

The research protocol received approval from the Institutional Ethical Committee of Vinayaka Mission's Sankarachariyar Dental College (Approval No.:VMSDC/IEC/Approval No.446) prior to the commencement of participant recruitment and sample collection. All procedures complied with the ethical principles outlined in the Declaration of Helsinki (2013 revision). Written informed consent was obtained from all participants before enrolment.

Study Population and Grouping (*Ex Vivo* Component)

Thirty complete maxillary denture wearers reporting for routine prosthodontic maintenance were recruited. Inclusion criteria included participants aged 50–75 years, wearing intact maxillary dentures fabricated from heat-polymerized polymethyl methacrylate (PMMA) for a minimum of six months. Subjects were excluded if they had used systemic or topical antibiotics or antifungals within four weeks, exhibited mucosal pathology, had relined or repaired dentures within the preceding three months, or had systemic diseases influencing oral microflora.

Participants were allocated to the three study groups (n = 10 per group) through a computer-generated random sequence to ensure unbiased distribution.

Group I (Control): Immersion in 100 mL distilled water.

Group II (Standard): Immersion in 100 mL of 0.2% chlorhexidine gluconate.

Group III (Test): Immersion in 100 mL aqueous *Camellia sinensis* extract standardized to its minimum inhibitory concentration (MIC).

Each participant immersed the denture once daily for 15 minutes over seven consecutive days. Following immersion, prostheses were rinsed with

potable water and reinserted. Adherence was recorded in participant logbooks and verified at review.

Preparation of *Camellia sinensis*

Extract

Authenticated *Camellia sinensis* (green tea) leaves were procured from a certified botanical source, shade-dried to constant weight, finely powdered and passed through a 40-mesh sieve prior to extraction. Extraction was carried out by aqueous Soxhlet technique using distilled water at 70 °C for eight hours to ensure exhaustive recovery of polyphenolic constituents (14). The extract was filtered through Whatman No. 1 filter paper to remove insoluble residues prior to concentration. The collected aqueous extract was concentrated under controlled reduced pressure using a rotary evaporator, followed by lyophilization to obtain a stable dry extract suitable for further analysis. A final extract yield of approximately 5% (w/w) relative to initial dry weight was obtained. The lyophilized material was stored in amber airtight vials at 4°C to preserve polyphenolic stability and minimize oxidative degradation. Initial phytochemical profiling verified the occurrence of major polyphenolic classes, including catechins, flavonoids and tannins, within the extract. Quantitative estimation of total catechin content was performed using UV-Visible spectrophotometry (Shimadzu UV-1800, Japan). A calibration curve (1–10 µg/mL) was established using analytical-grade catechin standards and the validated analytical wavelength (260 nm) was applied for all standardizations (15). The extraction and standardization procedure for the *Camellia sinensis* extract is illustrated in Figure 1. The workflow includes sequential collection, drying, pulverization and sieving, aqueous extraction, filtration, concentration, lyophilization, storage and standardization of the extract. These steps were designed to obtain a stable and standardized dry extract for subsequent antimicrobial and formulation studies. The workflow also demonstrates the progression from raw plant material to the analytically standardized catechin-containing extract.

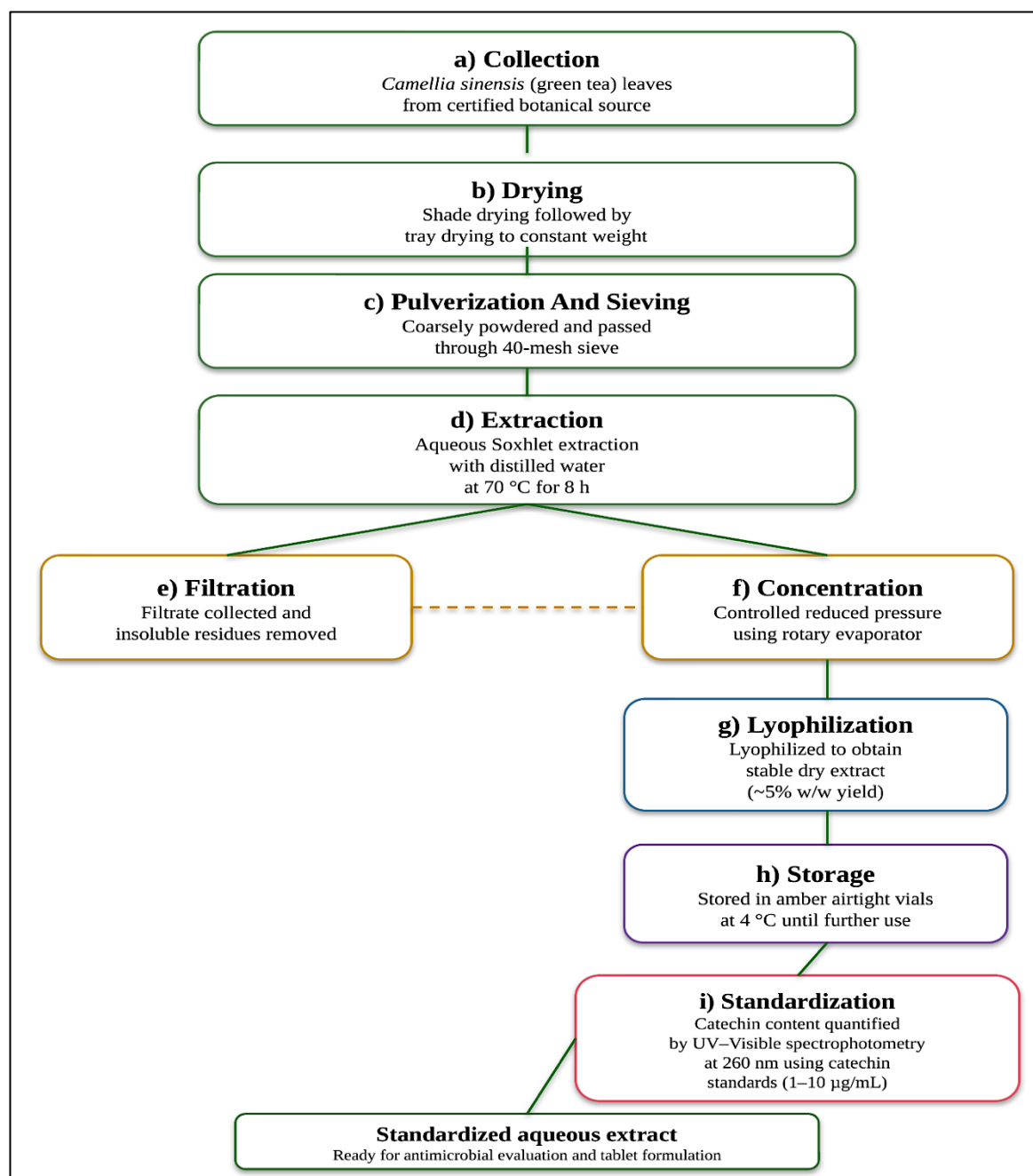


Figure 1: Workflow of Extraction and Standardization of *Camellia sinensis* (green tea) Extract

In Vitro Antimicrobial Evaluation

The antimicrobial activity was assessed against *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231) using standardized agar-well diffusion and broth dilution assays performed in accordance with established CLSI methodological frameworks (16).

Agar Well Diffusion

Microbial inocula were adjusted to a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL for bacteria and 1×10^6 CFU/mL for fungi, prior to plate inoculation. Agar wells of 6 mm in diameter were aseptically loaded with 50 µL

aliquots of extract solutions spanning concentrations between 10 and 60 µg/mL. Plates were incubated at 37 °C for 24 h for bacterial strains and 48 h for fungal isolates, after which diameters of growth-inhibition zones were recorded in millimeters.

Broth Microdilution

Serial twofold dilutions of the extract (500–0.9 µg/mL) were prepared in appropriate broth media (nutrient broth for *S. aureus* and Sabouraud broth for *C. albicans*). Each well of a 96-well plate received 100 µL of microbial inoculum and 100 µL of test solution. After incubation under controlled

assays, microbial growth was quantified by recording absorbance at 600 nm with a calibrated microplate spectrophotometer. The minimum inhibitory concentration (MIC) was defined as the lowest concentration at which complete inhibition of visible microbial growth was observed.

Formulation of Catechin-enriched Effervescent Tablets

Effervescent tablets containing 2% w/w standardized catechin extract were prepared by the direct compression technique. All excipients were dried to minimize residual moisture, passed through a 40-mesh sieve and blended in a double-cone blender under controlled low-humidity conditions to prevent premature effervescence. The acidic and alkaline components were initially blended separately and subsequently combined with the

extract and remaining excipients to ensure uniform mixing.

The composition of the catechin-enriched effervescent tablets is presented in Table 1. Catechin extract constituted 2% w/w of the formulation, while sodium bicarbonate, citric acid and tartaric acid formed the principal effervescent system. The 2% w/w value corresponds to the proportion of catechin extract incorporated into the final tablet formulation, whereas the approximately 5% w/w value reported above represents the extraction yield relative to the initial dry plant material. Microcrystalline cellulose, sodium starch glycolate and crospovidone were incorporated to support tablet integrity and rapid disintegration, whereas mannitol, aspartame, magnesium stearate and colloidal silicon dioxide served as diluent, sweetener, lubricant and glidant, respectively.

Table 1: Composition of Catechin-enriched Effervescent tablets

Ingredient	Function	Concentration (% w/w)
Catechin extract	Active phytoconstituent	2.0
Sodium bicarbonate	Effervescent base (alkali)	20.0
Citric acid anhydrous	Effervescent acid component	12.0
Tartaric acid	Effervescent acid component	12.0
Microcrystalline cellulose	Binder and filler	23.0
Sodium starch glycolate	Disintegrant	11.5
Crospovidone	Super-disintegrant	11.5
Aspartame	Sweetener	3.0
Mannitol	Diluent and mouthfeel enhancer	3.9
Magnesium stearate	Lubricant	1.0
Colloidal silicon dioxide	Glidant	0.1

Note: % w/w = percentage weight by weight.

The formulation composition was selected based on preliminary laboratory trials and established principles of effervescent tablet design. Sodium bicarbonate, citric acid and tartaric acid constituted the effervescent system. Microcrystalline cellulose functioned as a binder and filler, whereas sodium starch glycolate and crospovidone promoted rapid tablet disintegration. Mannitol served as a diluent and aspartame improved palatability. Magnesium stearate and colloidal silicon dioxide acted as lubricant and glidant, respectively.

The final composition was selected after preliminary trials based on tablet integrity, friability, disintegration behavior and effervescence characteristics.

Physicochemical Characterization

The formulated catechin-enriched effervescent tablets were subjected to comprehensive

physicochemical evaluation in accordance with the United States Pharmacopeia (USP) and International Council for Harmonization (ICH Q6A) guidelines for weight variation, hardness, friability, disintegration, effervescence time, pH, drug content uniformity and dissolution. Hardness and friability were evaluated to assess mechanical robustness, while disintegration and effervescence times were evaluated rapid reconstitution capability. The reconstituted solution pH was measured to confirm near-neutrality suitable for PMMA contact. Drug content was determined spectrophotometrically at 260 nm and dissolution studies (USP Apparatus I) were used to assess catechin release kinetics.

Spectroscopic Characterization

UV-visible Spectroscopy

Quantitative estimation and calibration of catechins were performed by UV-Visible

spectrophotometry at 260 nm using standard catechin solutions (1–10 µg/mL). Linearity, precision and accuracy of the method were confirmed ($R^2 \geq 0.998$; recovery 98–102%).

Fourier Transform Infrared (FTIR)

Spectroscopy

FTIR spectra of pure catechin, lyophilized extract and the formulated tablets were recorded using a FTIR spectrometer fitted with an ATR module. Spectral acquisition was carried out between 4000 cm^{-1} and 400 cm^{-1} at a resolution of 4 cm^{-1} to identify functional group signatures and assess any formulation-related interactions.

Cell Maintenance

L929 fibroblasts were maintained in DMEM supplemented with 10% fetal bovine serum and 1% antibiotic–antimycotic solution at 37°C in a humidified atmosphere containing 5% CO_2 .

Cytotoxicity Evaluation

Cytotoxicity was evaluated using the MTT colorimetric assay on L929 murine fibroblasts (17). A density of 1×10^4 cells per well was seeded in 96-well plates and allowed to attach over 24 h before exposure to serial concentrations (100–1000 µg/mL) of the reconstituted formulation. Following treatment, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 h to allow formazan formation. The resulting crystals were dissolved in dimethyl sulfoxide and absorbance was measured at 570 nm. Cell viability was computed relative to untreated control wells and corroborated with morphological assessments under an inverted microscope.

Clinical Ex Vivo Immersion Protocol

Microbial sampling was performed from the intaglio surface of each maxillary denture at baseline and after seven days of the immersion regimen. Samples were streaked onto blood agar plates for bacterial isolation and Sabouraud dextrose agar for fungal recovery (18), followed by

incubation at 37°C for 24 h and 48 h, respectively. Microbial burden was quantified by counting colony-forming units (CFU/mL) using a digital colony counter and values were $\log_{10}$ -transformed for statistical interpretation. All procedures were performed by a blinded microbiologist to minimize bias. The use of patient-derived dentures enabled evaluation under clinically relevant microbial conditions, although salivary dynamics and host immune interactions could not be reproduced under *ex vivo* conditions.

Statistical Analysis

All numerical outcomes were presented as mean values accompanied by their corresponding standard deviations. Differences among groups were analyzed using one-way ANOVA and when significant, Tukey's post hoc test was applied to identify pairwise contrasts. Statistical significance was defined at $p < 0.05$. All analyses were conducted using IBM SPSS Statistics, version 26.0.

Results

In Vitro Antimicrobial Evaluation

The standardized *Camellia sinensis* extract and the formulated effervescent solution demonstrated marked inhibitory effects against *Staphylococcus aureus* and *Candida albicans*. The antimicrobial findings obtained from the agar well diffusion assay is summarized in Table 2. The catechin-enriched effervescent tablet produced inhibition zones against both *Staphylococcus aureus* and *Candida albicans*, with larger inhibition zones observed at the higher tested concentration. The minimum inhibitory concentration values further supported the antimicrobial activity of the formulation against both microorganisms. Overall, the findings demonstrate a concentration-dependent inhibitory response under the evaluated *in vitro* conditions.

Table 2: *In Vitro* Antimicrobial Activity of Green Tea Extract and Catechin-enriched Effervescent Tablets against *S. aureus* and *C. albicans*

Test Material	Concentration (µg/mL)	<i>S. aureus</i> (mm) Mean ± SD	<i>C. albicans</i> (mm) Mean ± SD	MIC (µg/mL)	p-value
Aqueous extract	30	7.10 ± 0.18	6.85 ± 0.22	40	—
	60	14.35 ± 0.21	11.10 ± 0.17		
Effervescent tablet	250	11.35 ± 0.21	9.10 ± 0.14	0.9	<0.01
	500	12.95 ± 0.35	12.35 ± 0.21		

Note: MIC = minimum inhibitory concentration; SD = standard deviation; *S. aureus* = *Staphylococcus aureus*; *C. albicans* = *Candida albicans*; statistical significance was set at $p < 0.05$.

Minimum inhibitory concentration (MIC) analysis provided additional verification of the antimicrobial activity of the formulations. The standardized green tea extract demonstrated MIC value of 30 $\mu\text{g/mL}$ against *S. aureus* and 40 $\mu\text{g/mL}$ against *C. albicans*. The effervescent tablet solution exhibited MIC values of 0.9 $\mu\text{g/mL}$ against both *Staphylococcus aureus* and *Candida albicans*, indicating pronounced antimicrobial activity of the catechin-enriched formulation.

Physicochemical Evaluation

The formulated *Camellia sinensis* effervescent tablets exhibited a smooth, uniform surface with no visual evidence of cracking, lamination, or premature effervescence, confirming the absence of visible physical defects following tablet compression. The mean tablet thickness was 3.00 ± 0.08 mm, indicating dimensional uniformity among the prepared tablets.

The average tablet weight was 0.545 ± 0.009 g, with individual deviation well within $\pm 5\%$ of the mean, satisfying pharmacopeial weight variation limits.

The hardness of the tablets was 4.83 ± 0.22 kg/cm^2 , demonstrating an optimal balance between mechanical robustness and disintegration efficiency. Friability was minimal at 0.12 ± 0.02 %, confirming good compaction and absence of brittleness. The disintegration time was rapid, averaging 40 ± 2 seconds, while effervescence completed within 88 ± 3.2 seconds. These parameters collectively reflect a rapid-release effervescent system suitable for immediate aqueous dispersion. The reconstituted solution exhibited a mildly acidic pH (5.38 ± 0.10), which is

considered suitable for routine denture immersion applications and may contribute to maintaining compatibility with prosthetic materials.

Drug content uniformity testing confirmed consistent distribution of catechins within the formulation, with individual tablet assay values ranging between 98.2% and 101.6% of the labeled amount (mean = $100 \pm 2\%$).

The dissolution profile demonstrated rapid effervescence-mediated catechin release, with $92.15 \pm 1.12\%$ of the estimated catechin content released within 3 min. Near-complete catechin release was observed by 4 min.

The physicochemical characteristics of the catechin-enriched effervescent tablets are summarized in Table 3. The tablets demonstrated acceptable thickness and hardness with low friability, indicating adequate mechanical integrity. Rapid disintegration and effervescence were observed within the predefined acceptance limits, while the reconstituted solution exhibited a pH within the specified range. These characteristics collectively support the suitability of the formulation for rapid aqueous dispersion during denture immersion.

The dissolution behavior of the catechin-enriched effervescent tablets is illustrated in Figure 2. The tablet underwent visible effervescence following immersion in water, resulting in rapid dispersion of the formulation. The dissolution profile demonstrated rapid catechin release during the initial minutes of reconstitution, supporting the intended short-duration immersion application. The visual and dissolution findings together demonstrate the rapid dispersibility of the effervescent dosage form.

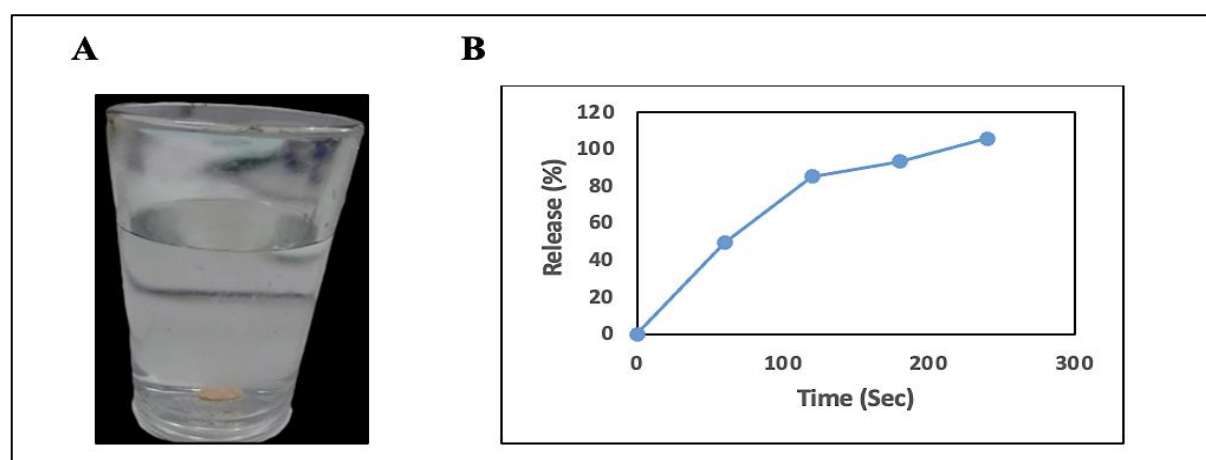


Figure 2: (A) Effervescent Tablet before and after Dissolution, (B) *In vitro* Dissolution profile of Catechin-enriched Effervescent Tablets

Table 3: Physicochemical Parameters of Catechin-enriched Effervescent Tablets

Parameter	Mean $\pm$ SD (n = 3)	Acceptance Criteria
Thickness (mm)	3.00 $\pm$ 0.08	Uniform
Hardness (kg/cm ²)	4.83 $\pm$ 0.22	3–6
Friability (%)	0.12 $\pm$ 0.02	$\leq$ 1%
Disintegration time (s)	40 $\pm$ 2	<90
Effervescence time (s)	88 $\pm$ 3.2	<120
pH of solution	5.38 $\pm$ 0.10	5.0–6.0

Note: SD = Standard Deviation, n = Number of Tablets Evaluated.

Spectroscopic Characterization

UV-Visible Spectrophotometry

The standard catechin calibration plot demonstrated strong linearity across the 1–10 $\mu\text{g/mL}$ concentration interval, with the regression equation $A = 0.058C + 0.012$ and a correlation coefficient of $R^2 = 0.998$, confirming the precision and reliability of the analytical method. The λ_{max} of both standard catechin and the formulated extract was observed at 260 nm, characteristic of $\pi \rightarrow \pi$ transitions of aromatic benzopyran rings

present in the catechin structure, confirming the characteristic absorption profile of catechin in the formulated extract. The peak detection data obtained from UV-visible spectroscopic analysis are presented in Table 4. The recorded absorbance values were associated with wavelength signals distributed across the evaluated spectral range. The highest absorbance values in the recorded peak-detection data occurred at 274.40 and 279.40 nm. These measurements provide additional numerical characterization of the spectral response of the catechin-enriched formulation.

Table 4: Peak Detection Data Obtained from UV-Visible Spectroscopic Analysis of the Catechin-enriched Formulation

Wavelength (nm)	Absorbance
396.70	0.000
390.90	0.000
385.00	0.000
377.80	0.000
367.80	0.000
356.80	0.000
341.10	0.002
322.50	0.001
318.80	0.002
301.10	0.002
299.30	0.002
279.40	0.005
274.40	0.005
263.10	0.004

Note: nm = nanometre.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis showed distinct spectral signatures corresponding to catechin and the *Camellia sinensis* extract. Prominent absorption bands were observed at 3362 cm^{-1} (O–H stretching vibrations of phenolic hydroxyl groups), 2923 cm^{-1} (aliphatic C–H stretching modes), 1614 cm^{-1} (aromatic ring C=C stretching transitions), 1452 cm^{-1} (O–H deformation band) and 1075 cm^{-1} (C–O stretching vibration). These spectral features remained evident in the effervescent formulation, with only slight band broadening, suggesting physical incorporation of the phytoconstituent without evidence of covalent interaction or structural

alteration. The lack of new or significantly shifted absorption bands supports the chemical compatibility of catechin with formulation excipients, including citric acid, sodium bicarbonate and microcrystalline cellulose. These findings support the spectral integrity of the phytoconstituent during tablet processing. The FTIR spectra of the investigated catechin samples are shown in Figure 3. Panel A represents pure catechin, whereas Panel B represents the green tea extract. Both spectra exhibited characteristic absorption bands corresponding to hydroxyl, aliphatic C–H, aromatic C=C and C–O functional groups. The major spectral features were retained across the samples, supporting preservation of the characteristic catechin spectral profile.

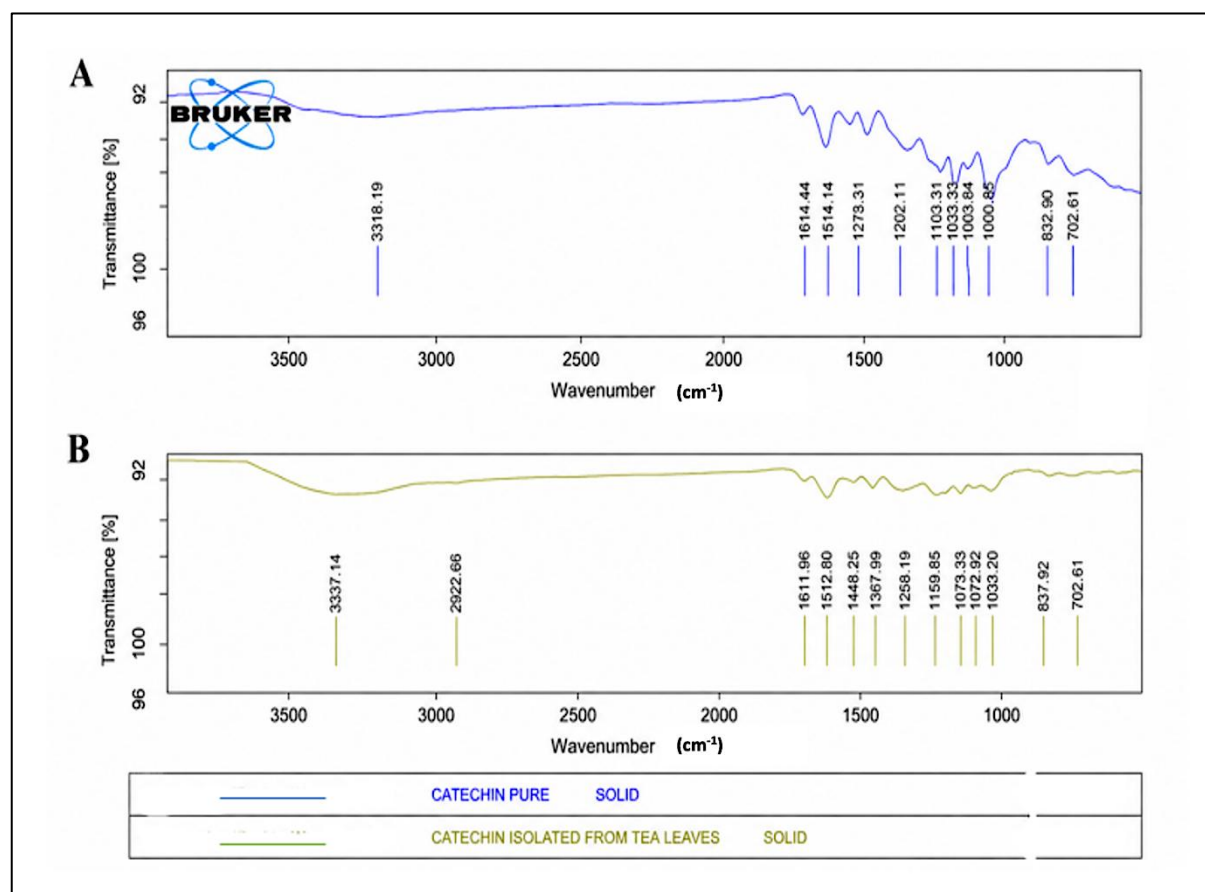


Figure 3: FTIR Spectra of Pure Catechin and Green Tea Extract. (A) Pure Catechin and (B) Green Tea Extract, Showing Characteristic Absorption Bands Corresponding to Hydroxyl, Aliphatic C-H, Aromatic C=C and C-O Functional Groups

Spectral Integrity Correlation

Spectral comparison between the extract and tablet confirmed retention of diagnostic catechin functional peaks, with no major spectral changes suggestive of new chemical interactions between the catechin-containing extract and the formulation excipients. The FTIR profile thus substantiates that the phytochemical profile of *Camellia sinensis* was conserved during

formulation, an essential criterion for phytopharmaceutical standardization. The characteristic absorption peaks identified by FTIR spectroscopy are summarized in Table 5. The major functional group regions showed comparable values across pure catechin, the extract and the effervescent tablet formulation. These findings indicate retention of the characteristic catechin-associated spectral features following formulation.

Table 5: Characteristic Absorption Peaks Identified by FTIR Spectroscopy

Sample	O-H (cm^{-1})	C-H (cm^{-1})	C=C (cm^{-1})	C-O-C (cm^{-1})
Pure catechin	3277	2966	1618–1500	1284–1220
Extract	3275	2967	1615–1504	1282–1218
Effervescent tablet	3273	2966	1616–1505	1283–1219

Note: O-H = hydroxyl group; C-H = carbon-hydrogen bond; C=C = carbon-carbon double bond; C-O-C = ether linkage; cm^{-1} = reciprocal centimetre.

Cytotoxicity Evaluation

The cytotoxic profile of the reconstituted effervescent solution was evaluated on L929 murine fibroblast cells using the MTT assay. The formulation preserved over 90% fibroblast

viability at concentrations up to 250 $\mu\text{g/mL}$, demonstrating a high degree of cytocompatibility within the evaluated exposure range. A concentration-dependent response was observed at higher doses, with an IC_{50} value of 467.3 $\mu\text{g/mL}$,

indicating low cytotoxic potential. Morphological examination under an inverted phase-contrast microscope revealed preserved fibroblast architecture, intact nuclei and absence of vacuolar degeneration across all test concentrations. These results substantiate the biocompatibility of the developed formulation for intraoral prosthetic applications. Representative microscopic images of L929 fibroblasts following exposure to the catechin-enriched effervescent tablet extract are

presented in Figure 4. The control cells showed the expected fibroblast morphology, while cells exposed to 400 and 1000 µg/mL maintained generally preserved cellular architecture. No prominent morphological features indicative of severe cellular damage was observed under the evaluated conditions. These morphological findings complement the quantitative MTT viability results and support the observed cytocompatibility of the formulation.

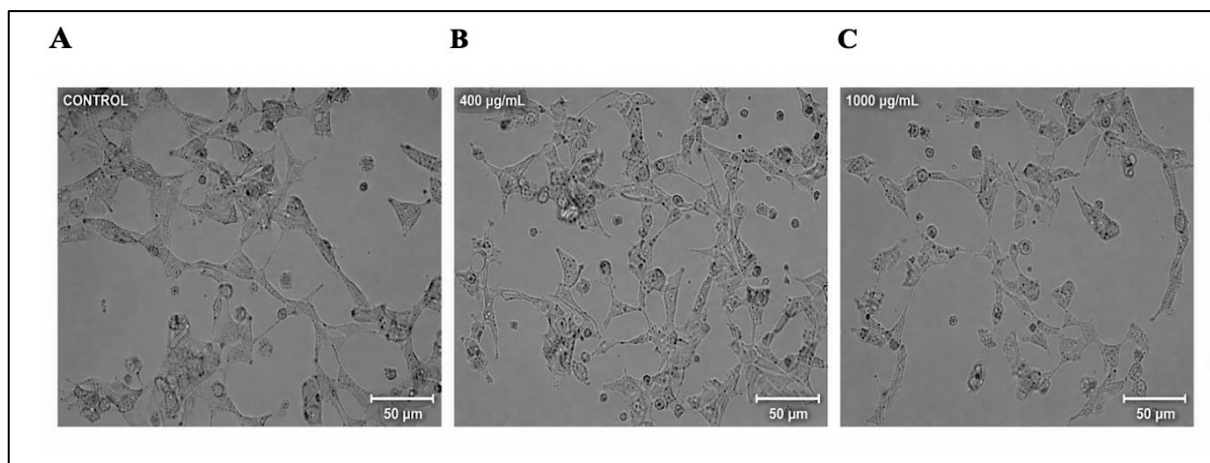


Figure 4: Morphology of L929 Fibroblasts Following Exposure to Catechin-enriched Effervescent Tablet Extract. (A) Control, (B) 400 µg/Ml and (C) 1000 µg/Ml. Representative Photomicrographs Show Preserved Cellular Morphology Across the Evaluated Concentrations (Scale Bars are Shown in Each Panel)

Ex Vivo Evaluation of Standardized Extract

Ex vivo analysis of dentures from participants after 7 days of immersion revealed a statistically significant reduction in microbial load across all treatment groups compared to baseline ($p < 0.05$). The green tea extract group demonstrated a 2.78 $\log_{10}$ reduction in *S. aureus* and a 2.25 $\log_{10}$ reduction in *C. albicans* counts, while chlorhexidine (0.2%) resulted in a marginally greater microbial reduction of 3.15 $\log_{10}$ units. Distilled water immersion produced negligible change (0.18 $\log_{10}$). Intergroup variations were assessed through one-way ANOVA and subsequent Tukey's post hoc testing verified statistically significant intergroup differences ($p < 0.05$). Microbial reduction was observed in all treatment groups following the 7-day immersion protocol, although the magnitude of reduction varied among the tested solutions.

Although chlorhexidine exhibited superior activity, the *Camellia sinensis* extract produced comparable microbial suppression under the evaluated *ex vivo* conditions. These findings

support the potential utility of *Camellia sinensis* extract as an adjunctive denture-cleansing agent.

Discussion

Antimicrobial Efficacy

The standardized catechin extract of *Camellia sinensis* demonstrated significant antibacterial and antifungal activity, with the effervescent formulation exhibiting a MIC of 0.9 µg/mL for both *Staphylococcus aureus* and *Candida albicans*, in contrast to 30 and 40 µg/mL observed for the plain extract. This enhanced antimicrobial efficacy may be attributed to the effervescence-mediated dispersion of the formulation, which facilitates rapid release and uniform distribution of active catechins throughout the aqueous medium.

These observations are consistent with previous reports that EGCG can interfere with metabolic and virulence-associated processes in *Streptococcus mutans* (19, 20). These findings support the cytocompatibility of the formulation under the evaluated *in vitro* conditions. Previous studies have also shown that polyphenol-rich extracts inhibit mixed bacterial and fungal biofilms through

oxidative membrane damage and suppression of quorum-sensing pathways (21).

The consistent pattern of inhibition observed across these studies supports the antimicrobial potential of catechin-based formulations. The findings of the present study closely parallel these previously reported outcomes, indicating that formulation into an effervescent dosage form may further improve the antimicrobial performance of catechins while preserving their inherent biological activity.

Physicochemical Characterization

The effervescent tablets displayed favorable mechanical and dissolution characteristics, including mean hardness of 4.83 kilograms per square centimeter, friability of 0.12 percent, disintegration within 40 seconds and complete effervescence by 88 seconds. The mildly acidic pH of 5.38 after effervescence indicates compatibility with routine denture immersion applications involving polymethyl methacrylate-based prostheses. Rapid disintegration and dissolution within 3–4 min indicate efficient aqueous dispersion characteristics.

These outcomes are consistent with evidence synthesized from previous studies demonstrating that effervescent denture cleansers with rapid dissolution and mildly acidic pH exhibit effective denture-cleaning performance while maintaining material stability (22). Comparable evidence indicates that prolonged exposure to effervescent denture-cleaning tablets can alter the colour and surface characteristics of acrylic splint materials, emphasizing the importance of evaluating material compatibility during prolonged use (23). A formulation study of an effervescent tablet containing plant-derived components also supports the feasibility of incorporating botanical constituents into an effervescent dosage form (24). The physicochemical attributes observed in the present study are therefore in agreement with these reports, indicating that effervescent systems optimized for rapid disintegration and controlled pH can provide both functional performance and favorable material compatibility.

Spectroscopic Identity and Structural Stability

The ultraviolet-visible spectrophotometric analysis demonstrated a distinct absorption maximum at 260 nanometers and excellent linearity ($R^2 = 0.998$), supporting the analytical reliability of

catechin quantification. Fourier transform infrared spectra of the formulated tablets retained the characteristic spectral bands associated with catechin, including broad hydroxyl stretching around 3362 reciprocal centimeters, aromatic carbon-carbon stretching at 1614 reciprocal centimeters and carbon-oxygen stretching at 1075 reciprocal centimeters. The absence of new or shifted peaks suggests preservation of the characteristic spectral features and indicates compatibility between catechins and formulation excipients.

Comparable observations have been reported for catechin-enriched polymeric systems, in which spectral profiles similar to those of the pure extract were retained following processing (25). Similar findings have also been documented for catechins incorporated into solid dispersions, where characteristic spectral and thermal signatures remained unchanged after formulation procedures (26). Existing evidence further supports the structural stability of catechins under mild processing conditions, which may be related to the hydrogen-bonding and conformational characteristics of catechin molecules described in molecular studies (27). These observations are consistent with the present findings and indicate retention of the characteristic catechin spectral features following formulation.

Cytotoxicity

The reconstituted effervescent solution maintained more than 90% fibroblast viability up to 250 $\mu\text{g/mL}$, with an IC_{50} value of 467.3 $\mu\text{g/mL}$, indicating relatively low cytotoxicity within the evaluated concentration range. These findings are consistent with earlier *in vitro* evidence demonstrating that green-tea-derived catechins have shown concentration-dependent effects on the viability of oral fibroblasts. Previous *in vitro* investigations have similarly reported sustained fibroblast viability across physiologically relevant concentrations, further supporting the favorable biocompatibility profile of catechin-based systems (28). Such concordant observations reinforce the suitability of these phytoconstituents for oral applications where preservation of soft tissue cellular integrity is essential.

Comparable findings have also been documented in epithelial models, where green-tea-derived catechin formulations maintained high cellular viability even at relatively elevated concentrations

and were associated with favorable biological responses, including attenuation of inflammatory activity and promotion of wound-healing behavior (29). In a non-oral epithelial cell model, EGCG has also been shown to reduce oxidative stress and preserve mitochondrial function under experimentally induced oxidative injury (11, 30). Collectively, these observations indicate that catechin-based formulations possess cytoprotective characteristics compatible with oral soft tissues and support their potential application in routine prosthodontic disinfection.

Ex vivo Antimicrobial Performance

The immersion of patient-derived dentures in standardized *Camellia sinensis* extract for seven consecutive days produced significant reductions in microbial load, amounting to reductions of 2.78 log₁₀ units in *Staphylococcus aureus* and 2.25 log₁₀ units in *Candida albicans*. These outcomes closely approached the 3.15 logarithmic reduction achieved with chlorhexidine, while maintaining comparable microbial suppression under the evaluated *ex vivo* conditions.

Comparable antimicrobial effects have been reported in studies evaluating effervescent tablets for denture biofilm control (31). These observations support the effectiveness of immersion-based disinfection strategies and provide context for the microbial reductions observed in the present investigation.

These findings are also consistent with clinical evidence supporting the antimicrobial benefits of phytotherapeutic agents in denture-associated infections. Previous clinical studies have demonstrated that *Camellia sinensis* extract can reduce *Candida* colonization in denture-stomatitis patients while maintaining favorable mucosal compatibility (12). This clinical concordance strengthens the translational relevance of the present findings and suggests that catechin-mediated microbial suppression may support oral hygiene maintenance in denture wearers.

Comparable findings have also been reported in experimental denture-cleansing studies. Effervescent denture tablets have demonstrated antimicrobial activity against multispecies biofilms, while botanical denture-cleansing formulations have shown antifungal activity together with evaluation of their effects on denture material properties (32, 33). These findings parallel the present immersion results, collectively

indicating that catechin-based formulations can deliver clinically meaningful antimicrobial efficacy while demonstrating favorable cytocompatibility in the present investigation.

Future translational development of such formulations would require compliance with applicable quality and safety standards governing oral hygiene and denture-cleansing products.

Considering the combined antimicrobial, physicochemical and cytocompatibility outcomes, the overall evidence did not substantiate the null hypothesis, which proposed equivalence between the catechin-based formulations and the standard control solutions.

Limitations

The present study has certain limitations that should be considered while interpreting the findings. Although the antimicrobial efficacy and formulation characteristics were evaluated through *in vitro* and *ex vivo* analyses, long-term clinical performance and patient-based outcomes were not assessed. Detailed chromatographic characterization, post-compression catechin recovery analysis and advanced formulation optimization approaches were beyond the scope of the present investigation. The antimicrobial evaluation primarily focused on planktonic microorganisms, while biofilm-specific assays and additional oral cell-line studies were not included. Furthermore, the *ex vivo* model could not fully reproduce the complexity of the oral environment, including salivary dynamics and host immune interactions. Long-term packaging stability and post-formulation antioxidant activity also warrant further investigation.

Clinical Significance

The developed *Camellia sinensis* effervescent tablet provides a plant-derived approach for denture cleansing with demonstrated antimicrobial activity and favorable cytocompatibility. Its rapid dissolution characteristics, ease of use and compatibility with routine denture immersion protocols make it a promising adjunct for daily prosthodontic hygiene. Incorporation of naturally derived catechins into an effervescent dosage form may offer an alternative strategy for supporting denture hygiene and maintaining oral health in denture wearers.

Conclusion

The present investigation demonstrates the successful development of a catechin-enriched effervescent denture-cleansing formulation based on standardized *Camellia sinensis* extract. The formulation exhibited acceptable physicochemical characteristics, retained the characteristic spectral features of catechin, demonstrated antimicrobial activity against the tested microorganisms and maintained favorable fibroblast viability within the evaluated concentration range. The *ex vivo* immersion study further demonstrated a substantial reduction in microbial load following treatment, with the observed reduction approaching that of chlorhexidine under the conditions investigated. The findings from the physicochemical, antimicrobial, cytocompatibility and *ex vivo* evaluations provide an overall assessment of the developed formulation under the conditions investigated.

The findings obtained in the present study provide a basis for further investigation of catechin-enriched effervescent formulations for denture cleansing. However, the present evidence remains confined to the experimental *in vitro* and *ex vivo* conditions and therefore does not establish clinical efficacy. Further studies involving detailed catechin characterization, biofilm-specific antimicrobial assessment, long-term formulation stability and clinical evaluation are required to determine whether the observed findings can be reproduced under more clinically relevant conditions.

Abbreviations

ATCC: American Type Culture Collection, CLSI: Clinical and Laboratory Standards Institute, DMEM: Dulbecco's modified Eagle medium, IC₅₀: Half Maximal Inhibitory Concentration, MIC: Minimum Inhibitory Concentration, MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide, UV: Ultraviolet.

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Author Contributions

Shanthi Chinnadurai: Conceptualization, Methodology, Investigation, Data Analysis, Manuscript Preparation, Review, Bhuvanesh Kumar Dharani Vidya: Investigation, Data Collection, Manuscript Review, Selvamani Chellamuthu: Investigation, Data Collection, Manuscript Review, Ganesamoorthy R: Supervision, Manuscript Review, Mohamed Adhil K: Investigation, Data Collection, Manuscript Review, Priyadharshini R, Sathish P: Investigation, Data Collection, Manuscript Review, Kalaivani T: Investigation, Data Collection, Manuscript Review. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest related to this study.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Artificial Intelligence (AI) Assistance

Generative AI tools were not used in the study design, experimental procedures, data collection, analysis, or interpretation of results. AI-assisted tools were used during figure preparation solely to add and format descriptive labels, panel identifiers, axis titles and scale-bar annotations to existing experimental figures. No scientific data or experimental content were generated or altered using AI. All scientific content and conclusions were reviewed and verified by the authors.

Ethics Approval

The study protocol was approved by the Institutional Ethical Committee of Vinayaka Mission's Sankarachariyar Dental College (Approval No: VMSDC/IEC/Approval No.446).

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