Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 22 May 2026
Revised 29 June 2026
Accepted 10 July 2026

Natural Resources for Human Health 2026, Vol. 6 (12s): 1421–1433

KEYWORDS:
Periodontitis, polyphenols, epigallocatechin-3-gallate, quercetin, curcumin, chlorhexidine, Porphyromonas gingivalis, cytokines, biofilm, simulation.

Antimicrobial and Anti-Inflammatory Effects of Plant-derived Polyphenols (EGCG, Quercetin and Curcumin) against Periodontal Pathogens: an In Vitro Experimental Sample Study using a Simulated Dataset

Polyphenols against Periodontal Pathogens–Sample Study (Simulated Data)

Arbaz Sajjad[1*], Jestin Chellian[1], Arul Kiruba N. Mary[2], Mary Minolin T[2], Rajan Rajabalaya[1], Srijeeta Maity[3]

[1]Pengiran Anak Puteri Rashidah Sa'adatul Bolkiah Institute of Health Sciences, Universiti Brunei Darussalam, Gadong, BE1410 Brunei Darussalam
[2]Saveetha College of Nursing, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Kuthambakkam, Tamil Nadu 602105, India
[3]Department of Oral Medicine and Radiology, Kalinga Institute of Dental Sciences, Kalinga Institute of Industrial Technology (KIIT) Deemed to be University, Bhubaneswar, Odisha, India
Corresponding Author: Dr. Arbaz Sajjad, Faculty of Dentistry, Pengiran Anak Puteri Rashidah Sa'adatul Bolkiah Institute of Health Sciences, Universiti Brunei Darussalam, Gadong, BE1410 Brunei Darussalam
Email: arbaz.sajjad@ubd.edu.bn

ABSTRACT:

Background: Periodontitis is a dysbiotic, host-mediated inflammatory disease, and chlorhexidine, the reference antiseptic adjunct, has limited anti-inflammatory activity and measurable cytotoxicity. Plant-derived polyphenols offer combined antimicrobial and host-modulating properties. This sample study sets out the design, analysis and reporting framework for an in vitro comparison of epigallocatechin-3-gallate (EGCG), quercetin and curcumin against periodontal pathogens and inflammatory mediators.

Methods: The framework assumes 200 subgingival plaque samples from patients with Stage II–IV periodontitis, randomly allocated to five groups (n = 40 each): vehicle control, EGCG, quercetin, curcumin and 0.12% chlorhexidine (positive control). Outcomes were minimum inhibitory and bactericidal concentrations (MIC/MBC), agar-well zones of inhibition, crystal-violet biofilm inhibition, IL-1β, IL-6, TNF-α and PGE2 in LPS-stimulated gingival fibroblasts before and 24 h after exposure (ELISA), and fibroblast viability (MTT). All outcome values in this version were computer-generated (random seed 2026) around literature-plausible ranges; no laboratory measurements were made. Data were analysed with Kruskal–Wallis, Welch ANOVA with Games–Howell post hoc tests and paired t-tests.

Results (simulated, illustrative only): In the simulated dataset, chlorhexidine produced the lowest MIC (median 3.9 μg/mL), the largest zone of inhibition (20.8 ± 1.8 mm) and the greatest biofilm inhibition (72.9 ± 8.3%), but the lowest fibroblast viability (68.6 ± 5.4%) and the smallest cytokine reductions (≈19–20%). All three polyphenols reduced IL-

Natural Resources for Human Health
eISSN: 2583-1194

1β, IL-6, TNF-α and PGE2 from baseline (all $p < 0.001$), by 37–46%, while viability remained above 89%. EGCG showed the greatest anti-inflammatory and antibiofilm effect among the polyphenols; quercetin had the highest MIC (median 125 μg/mL). The vehicle control showed no significant change.

Conclusion: This sample study provides a complete, reproducible analytic and reporting template. The simulated pattern mirrors the hypothesis that polyphenols trade some antimicrobial potency for superior host modulation and biocompatibility relative to chlorhexidine. No inference about the true effects of these compounds may be drawn until the simulated values are replaced with actual laboratory measurements.

1. INTRODUCTION

Periodontitis is a chronic, multifactorial inflammatory disease of the tooth-supporting tissues, characterised by progressive loss of clinical attachment and alveolar bone and classified since 2017 by stage and grade [1,2]. Severe periodontitis affects roughly one in ten adults worldwide and is among the most prevalent chronic conditions of humankind [3,4]. Beyond tooth loss, it is linked through systemic inflammation with cardiometabolic and other comorbidities [5,6], and individual susceptibility is shaped by behavioural, systemic and genetic risk factors [7,8].

The disease is initiated by a dysbiotic subgingival biofilm. Classical work described 'red' and 'orange' microbial complexes [9], and current models emphasise polymicrobial synergy and dysbiosis in which keystone organisms such as *Porphyromonas gingivalis* subvert host immunity [10,11]. *P. gingivalis*, *Tannerella forsythia* [12], *Fusobacterium nucleatum* [13] and *Aggregatibacter actinomycetemcomitans* [14] are the principal pathogens studied in vitro, and their organisation within a biofilm confers marked tolerance to antimicrobials [15].

Tissue destruction, however, is largely host-mediated. Bacterial products trigger the release of IL-1β, IL-6 and TNF-α and the COX-2/PGE2 pathway, which drive matrix degradation and osteoclastic bone resorption [16–19]; oxidative stress amplifies this cascade [20]. An ideal adjunct would therefore act on both the microbial and the inflammatory arms of the disease.

Mechanical debridement remains the cornerstone of therapy [21,22], supplemented where appropriate by regenerative materials and biologics [23,24] and by adjuncts such as diode lasers for oral soft-tissue management [25,26]. Systemic and local antimicrobials offer additional benefit in selected cases [27,28], and combined regimens exploit antimicrobial synergy [29], but resistance among periodontal microbiota is a growing concern [30]. Chlorhexidine is the reference antiseptic [31,32] and continues to be used as the comparator in trials of herbal and non-antibiotic adjuncts during scaling and root planing [33]. Its drawbacks include cytotoxicity towards fibroblasts and other host cells [34], possible induction of reduced susceptibility [35] and minimal host-modulating activity, which has driven interest in host-modulation therapy [36].

Natural products remain a major source of new therapeutic agents [37]. Polyphenols are abundant plant secondary metabolites with antioxidant, anti-inflammatory and antimicrobial activity [38–40], exerting antibacterial effects through membrane disruption, enzyme inhibition and interference with virulence factors [41–43]. Several reviews support their potential in periodontal inflammation [44–46]. EGCG, the principal green-tea catechin [47], inhibits *P. gingivalis* growth, adherence and biofilm formation [48,49], protects epithelial defensins [50] and green-tea intake is inversely associated with periodontal disease [51]. Curcumin inhibits periodontopathic bacteria and gingipain activity [52,53] and down-regulates inflammatory mediators in vitro and in vivo [54–56], although its bioavailability is poor [57]. Quercetin inhibits *P. gingivalis* and *A. actinomycetemcomitans* [58], reduces experimental periodontal bone loss [59,60] and modulates NF-κB-dependent cytokine signalling [61,62]. Related polyphenol fractions likewise dampen macrophage cytokine responses to periodontopathogen LPS [63,64].

Few studies have compared these three polyphenols head-to-head against a panel of clinical periodontal isolates while simultaneously quantifying their effects on inflammatory mediators and host-cell viability, with chlorhexidine as the benchmark. The aim of this sample study was to build and demonstrate the complete experimental, analytic and reporting framework for such a comparison. Because the outcome data in this version are simulated, the results section illustrates how findings would be presented and must not be interpreted as evidence. The working hypothesis is that EGCG, quercetin and curcumin are less potent antimicrobials than chlorhexidine but produce greater suppression of IL-1β, IL-6, TNF-α and PGE2 with better preservation of fibroblast viability.

Natural Resources for Human Health
eISSN: 2583-1194

2. MATERIALS AND METHODS

2.1 Nature of this sample study

This document is a sample (template) study. The protocol below describes the intended laboratory procedures. The accompanying dataset (n = 200) was generated with random numbers (seed 2026) around ranges considered plausible from the published literature, in order to develop the statistical analysis plan, test the analysis code and design the results tables and figures. No patient was recruited and no laboratory assay was performed for this version. All values reported in Section 3 are therefore simulated and illustrative. When actual measurements become available, the same analysis pipeline is to be re-run and every number, table and figure replaced.

2.2 Study design and samples

The planned design is an in vitro experimental study using subgingival plaque samples from 200 systemically healthy adults with periodontitis (Stage II–IV, 2017 AAP/EFP classification) [1,2]. For each participant, age, sex, stage, mean probing pocket depth (PPD), mean clinical attachment loss (CAL) and bleeding on probing (BOP, % of sites) are to be recorded. Samples are to be collected with sterile paper points from the deepest pocket after supragingival plaque removal and transported in reduced transport fluid. Ethical approval and written informed consent must be obtained before any sample collection [details to be inserted].

2.3 Isolation and identification of pathogens

Samples are to be cultured anaerobically (80% N_2, 10% H_2, 10% CO_2, 37 °C) on supplemented blood agar and selective media. The predominant isolate from each sample (*P. gingivalis*, *F. nucleatum*, *T. forsythia* or *A. actinomycetemcomitans*) is to be identified by colony morphology, biochemical profile and confirmatory species-specific PCR.

2.4 Test agents and allocation

Samples are to be randomly allocated by computer-generated sequence to five groups (n = 40 each): (i) vehicle control (culture medium with ≤0.5% DMSO), (ii) EGCG, (iii) quercetin, (iv) curcumin (each ≥95% purity, dissolved in DMSO and diluted in medium) and (v) 0.12% chlorhexidine digluconate (positive control). Laboratory personnel performing the assays and the analyst are to be blinded to group identity using coded tubes.

2.5 Minimum inhibitory and bactericidal concentrations

MIC is to be determined by broth microdilution in supplemented Brucella broth using two-fold serial dilutions (1.95–1000 μg/mL) according to CLSI M11 for anaerobes [65,66]. The MIC is the lowest concentration without visible growth after 48–72 h. For MBC, 10 μL from each clear well is to be subcultured on blood agar; the MBC is the lowest concentration producing ≥99.9% kill. An MBC/MIC ratio ≤ 4 is interpreted as bactericidal.

2.6 Agar well diffusion

Standardised inocula (0.5 McFarland) are to be spread on blood agar, 6-mm wells cut and filled with 50 μL of each agent, and zones of inhibition measured in millimetres after anaerobic incubation, including the well diameter (a value of 6.0 mm therefore denotes no inhibition) [67].

2.7 Biofilm inhibition assay

Biofilms are to be formed in 96-well plates for 48 h in the presence of sub-MIC concentrations of each agent, stained with 0.1% crystal violet, solubilised in 30% acetic acid and read at 570 nm [68]. Inhibition is expressed as percentage reduction in optical density relative to untreated wells.

2.8 Fibroblast culture, LPS stimulation and cytokine measurement

Primary human gingival fibroblasts (passages 3–6) are to be stimulated with *P. gingivalis* LPS (1 μg/mL) for 24 h. Supernatants collected at this point constitute the baseline value. Cells are then exposed to each agent at a non-toxic concentration for 24 h, after which supernatants are collected again. IL-1β, IL-6, TNF-α and PGE2 are to be quantified in duplicate with commercial ELISA kits and expressed in pg/mL. Percentage reduction is calculated as (baseline − post-treatment)/baseline × 100.

2.9 Cell viability

Viability of fibroblasts after 24 h exposure is to be measured by the MTT assay [69] and expressed as a percentage of untreated cells.

2.10 Sample size

Sample size was set a priori with G*Power 3.1 [70]. For a one-way ANOVA across five groups with a medium effect size ($f = 0.25$), $\alpha = 0.05$ and power 0.80, 200 samples (40 per group) exceed the required minimum and allow for assay failures.

2.11 Statistical analysis

Continuous data are summarised as mean ± standard deviation (SD) and ordinal MIC/MBC data as median (interquartile range, IQR). Baseline comparability was assessed with one-way ANOVA and chi-square tests. MIC and MBC across active agents were compared with the Kruskal–Wallis test and Bonferroni-corrected Mann–Whitney U tests. Because the vehicle control has no variance for zone of inhibition, zones were compared with the Kruskal–Wallis test, with Games–Howell pairwise comparisons among the active agents. Biofilm inhibition, viability and cytokine percentage reduction were compared with Welch's ANOVA (Levene's test indicated unequal variances) followed by Games–Howell post hoc tests; partial η^2 is reported as effect size. Within-group changes from baseline were tested with paired t-tests. Two-way ANOVA (group × pathogen) was used to check whether effects differed by isolate, and ANCOVA adjusted for age as a sensitivity analysis. Associations were assessed with Spearman's ρ. Two-sided $p < 0.05$ was considered significant. Analyses were performed in Python 3 (SciPy, statsmodels, pingouin).

3. RESULTS (SIMULATED DATA – ILLUSTRATIVE ONLY)

Participant characteristics. The simulated cohort had a mean age of 43.5 ± 10.5 years (range 25–70); 103 (51.5%) were male, and 75, 99 and 26 samples were classified as Stage II, III and IV, respectively. The predominant isolates were *P. gingivalis* (n = 64), *F. nucleatum* (n = 52), *T. forsythia* (n = 46) and *A. actinomycetemcomitans* (n = 38). Sex, stage, PPD, CAL, BOP and pathogen distribution did not differ between groups (Table 1). Mean age was lower in the vehicle-control group ($p = 0.021$), but adjusting for age did not alter any group effect (ANCOVA, age $p \geq 0.26$).

Table 1. Baseline characteristics of participants and samples by treatment group (simulated data)

Variable	Vehicle control (n=40)	EGCG (n=40)	Quercetin (n=40)	Curcumin (n=40)	CHX 0.12% (n=40)	p
Age (years), mean ± SD	38.9 ± 10.4	44.9 ± 10.8	44.5 ± 9.5	43.2 ± 11.3	46.2 ± 9.4	0.021
Male, n (%)	21 (52)	23 (58)	17 (42)	23 (58)	19 (48)	0.605
Stage II, n (%)	10 (25)	17 (42)	13 (32)	16 (40)	19 (48)	0.538
Stage III, n (%)	23 (58)	17 (42)	22 (55)	21 (52)	16 (40)	
Stage IV, n (%)	7 (18)	6 (15)	5 (12)	3 (8)	5 (12)	
Mean PPD (mm), mean ± SD	5.7 ± 0.9	5.5 ± 1.1	5.5 ± 0.8	5.5 ± 1.1	5.5 ± 1.0	0.881
Mean CAL (mm), mean ± SD	6.6 ± 1.1	6.3 ± 1.3	6.3 ± 1.0	6.3 ± 1.2	6.2 ± 0.9	0.614
BOP (%), mean ± SD	52.8 ± 12.5	53.6 ± 15.4	56.2 ± 15.8	52.0 ± 13.9	55.0 ± 16.1	0.712
Porphyromonas gingivalis, n (%)	13 (32)	13 (32)	14 (35)	11 (28)	13 (32)	0.814
Fusobacterium nucleatum, n (%)	8 (20)	12 (30)	11 (28)	11 (28)	10 (25)	

Variable	Vehicle control (n=40)	EGCG (n=40)	Quercetin (n=40)	Curcumin (n=40)	CHX 0.12% (n=40)	p
Tannerella forsythia, n (%)	9 (22)	6 (15)	7 (18)	11 (28)	13 (32)	
Aggregatibacter actinomycetemcomitans, n (%)	10 (25)	9 (22)	8 (20)	7 (18)	4 (10)	

Values are mean ± SD or n (%). p-values from one-way ANOVA (continuous) or chi-square test (categorical). PPD, probing pocket depth; CAL, clinical attachment loss; BOP, bleeding on probing; CHX, chlorhexidine.

Antimicrobial activity. MIC and MBC differed significantly among the active agents (Kruskal–Wallis H = 114.9 and 111.5, both $p < 0.001$; Table 2). Chlorhexidine had the lowest median MIC (3.9 μg/mL) and MBC (7.8 μg/mL). EGCG and curcumin shared a median MIC of 62.5 μg/mL and MBC of 125 μg/mL (no significant difference, adjusted $p = 1.00$), whereas quercetin was less potent (median MIC 125 μg/mL, MBC 250 μg/mL; adjusted $p < 0.001$ versus both). All MBC/MIC ratios were ≤ 4, indicating bactericidal activity. The ranking was consistent across the four pathogens (Figure 1).

Table 2. Minimum inhibitory (MIC) and bactericidal (MBC) concentrations (μg/mL) of the test agents (simulated data)

Parameter	EGCG	Quercetin	Curcumin	CHX 0.12%	p
MIC, all isolates	62.5 (31.25–62.5)	125 (109.375–125)	62.5 (31.25–62.5)	3.9 (1.95–3.9)	<0.001
Porphyromonas gingivalis	62.5 (31.25–125)	125 (62.5–250)	62.5 (31.25–62.5)	1.95 (1.95–7.8)	<0.001
Fusobacterium nucleatum	62.5 (31.25–125)	125 (62.5–125)	62.5 (31.25–125)	3.9 (1.95–7.8)	<0.001
Tannerella forsythia	62.5 (31.25–125)	125 (62.5–250)	31.25 (31.25–62.5)	3.9 (1.95–7.8)	<0.001
Aggregatibacter actinomycetemcomitans	62.5 (31.25–125)	125 (62.5–250)	62.5 (62.5–125)	2.925 (1.95–3.9)	0.003
MBC, all isolates	125 (125–250)	250 (250–500)	125 (125–250)	7.8 (3.9–9.75)	<0.001
Porphyromonas gingivalis	125 (62.5–500)	250 (125–1000)	125 (62.5–125)	7.8 (3.9–31.2)	<0.001
Fusobacterium nucleatum	125 (62.5–250)	250 (250–500)	250 (125–500)	7.8 (3.9–31.2)	<0.001
Tannerella forsythia	125 (62.5–500)	500 (250–1000)	125 (62.5–250)	7.8 (3.9–15.6)	<0.001
Aggregatibacter actinomycetemcomitans	125 (62.5–500)	250 (125–500)	250 (125–250)	5.85 (3.9–7.8)	0.006
MBC/MIC ratio ≤ 4, n (%)	40 (100)	40 (100)	40 (100)	40 (100)	—

All isolates: median (IQR); by pathogen: median (range). p, Kruskal–Wallis test across active agents. The vehicle control showed no antimicrobial activity and is omitted. Bonferroni-corrected Mann–Whitney: EGCG vs curcumin $p = 1.00$; quercetin vs EGCG and vs curcumin $p < 0.001$; CHX vs each polyphenol $p < 0.001$.

Natural Resources for Human Health
eISSN: 2583-1194

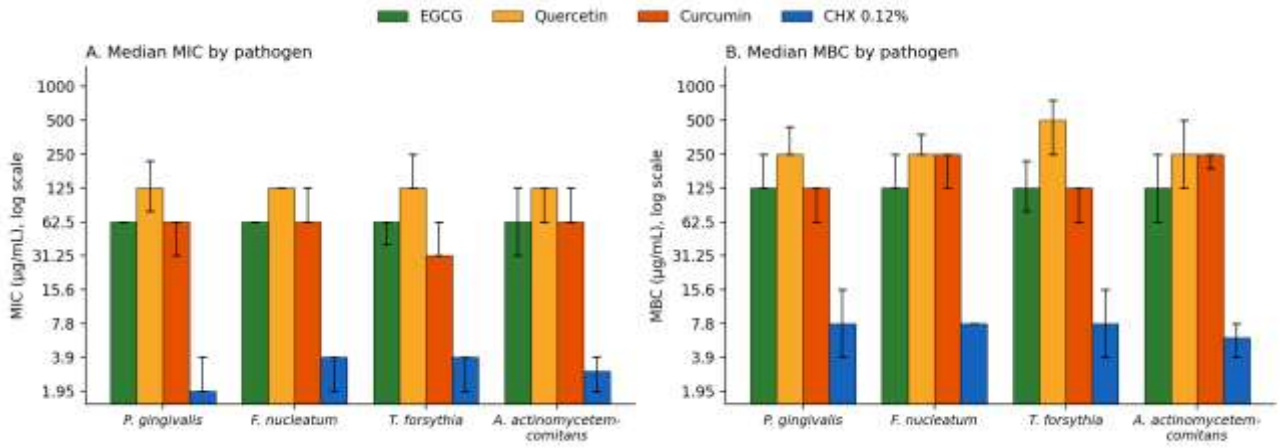

Figure 1. Median (IQR) minimum inhibitory concentration (A) and minimum bactericidal concentration (B) of EGCG, quercetin, curcumin and chlorhexidine 0.12% against each periodontal pathogen, log scale (simulated data). The vehicle control showed no antimicrobial activity.

Zone of inhibition and biofilm inhibition. Zones differed across groups ($H = 155.0$, $p < 0.001$; Table 3, Figure 2A–B). Chlorhexidine produced the largest zones (20.8 ± 1.8 mm), followed by EGCG (15.4 ± 2.4 mm), curcumin (14.4 ± 2.1 mm) and quercetin (13.5 ± 2.7 mm); the vehicle showed no inhibition. EGCG zones exceeded those of quercetin ($p = 0.008$). Biofilm inhibition differed markedly (Welch $F = 897.2$, $p < 0.001$, partial $\eta^2 = 0.89$): chlorhexidine 72.9 ± 8.3%, EGCG 45.6 ± 8.4%, curcumin 42.3 ± 9.5%, quercetin 38.4 ± 8.2% and vehicle 3.4 ± 2.9%. EGCG was superior to quercetin ($p = 0.002$). Neither pathogen ($p = 0.79$) nor the group × pathogen interaction ($p = 0.64$) was significant in two-way ANOVA.

Table 3. Zone of inhibition, biofilm inhibition and fibroblast viability by group (simulated data)

Outcome	Vehicle control (n=40)	EGCG (n=40)	Quercetin (n=40)	Curcumin (n=40)	CHX 0.12% (n=40)	p
Zone of inhibition (mm)	6.0 ± 0.0	15.4 ± 2.4	13.5 ± 2.7	14.4 ± 2.1	20.8 ± 1.8	<0.001[a]
Biofilm inhibition (%)	3.4 ± 2.9	45.6 ± 8.4	38.4 ± 8.2	42.3 ± 9.5	72.9 ± 8.3	<0.001[b]
Cell viability (%)	97.9 ± 1.9	92.1 ± 3.1	91.4 ± 4.6	89.1 ± 4.7	68.6 ± 5.4	<0.001[b]

Mean ± SD. [a]Kruskal–Wallis test (vehicle zone = 6.0 mm well diameter, no variance). [b]Welch ANOVA. Games–Howell: CHX differed from all groups for every outcome ($p < 0.001$); EGCG > quercetin for zone ($p = 0.008$) and biofilm ($p = 0.002$); curcumin < EGCG for viability ($p = 0.014$).

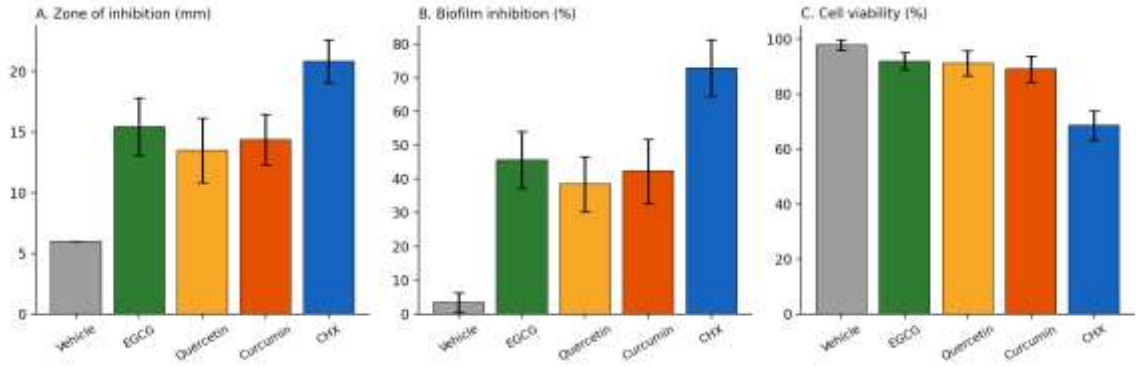

Figure 2. Zone of inhibition (A), biofilm inhibition (B) and fibroblast viability (C) by treatment group (mean ± SD; simulated data). The vehicle zone of 6.0 mm equals the well diameter (no inhibition). CHX, chlorhexidine 0.12%.

Anti-inflammatory effects. Baseline cytokine levels were similar across groups (Table 4). All three polyphenols and chlorhexidine reduced IL-1β, IL-6, TNF-α and PGE2 from baseline (paired t-tests, all $p < 0.001$; Figure 3), whereas the vehicle showed no significant change. Percentage reductions differed among groups for every mediator (Welch ANOVA, all $p < 0.001$, partial η^2 0.86–0.89; Figure 4). EGCG produced the largest reductions (IL-1β 43.9%, IL-6 46.0%, TNF-α 44.0%, PGE2 45.8%), followed by curcumin (40.1–40.7%) and quercetin (37.0–37.7%); chlorhexidine achieved only 19.0–20.0%. Games–Howell tests (Table 5) showed EGCG > curcumin for all four mediators ($p \leq 0.005$), curcumin > quercetin for IL-1β ($p = 0.008$) and TNF-α ($p = 0.017$) but not IL-6 ($p = 0.054$) or PGE2 ($p = 0.181$), and every polyphenol > chlorhexidine ($p < 0.001$). Among polyphenol-treated samples, biofilm inhibition correlated weakly with IL-1β reduction ($\rho = 0.22$, $p = 0.017$).

Table 4. Inflammatory mediators in LPS-stimulated gingival fibroblasts before and after 24 h exposure (simulated data)

Mediator	Vehicle control (n=40)	EGCG (n=40)	Quercetin (n=40)	Curcumin (n=40)	CHX 0.12% (n=40)	p (between groups)
IL-1β baseline (pg/mL)	431.0 ± 77.9	412.0 ± 64.1	387.7 ± 65.9	419.8 ± 69.2	436.0 ± 65.3	
IL-1β post-treatment (pg/mL)	422.3 ± 77.7	231.6 ± 41.5	243.6 ± 47.2	248.7 ± 44.1	347.8 ± 58.7	
IL-1β paired p (baseline vs post)	0.125	<0.001	<0.001	<0.001	<0.001	
IL-1β reduction (%)	1.8 ± 7.7	43.9 ± 3.6	37.3 ± 4.4	40.7 ± 4.3	20.0 ± 7.5	<0.001
IL-6 baseline (pg/mL)	615.9 ± 72.4	621.4 ± 73.3	602.5 ± 108.2	636.0 ± 92.8	613.0 ± 107.5	
IL-6 post-treatment (pg/mL)	615.0 ± 105.0	336.3 ± 57.4	375.5 ± 74.1	377.9 ± 68.1	497.7 ± 104.6	
IL-6 paired p (baseline vs post)	0.926	<0.001	<0.001	<0.001	<0.001	
IL-6 reduction (%)	0.4 ± 9.9	46.0 ± 5.1	37.7 ± 4.4	40.7 ± 5.2	19.0 ± 7.8	<0.001
TNF-α baseline (pg/mL)	334.1 ± 45.8	359.9 ± 54.6	345.6 ± 57.6	358.3 ± 63.9	353.2 ± 58.7	
TNF-α post-treatment (pg/mL)	328.4 ± 52.4	202.0 ± 38.2	217.7 ± 40.2	214.9 ± 43.0	282.6 ± 48.7	
TNF-α paired p (baseline vs post)	0.115	<0.001	<0.001	<0.001	<0.001	
TNF-α reduction (%)	1.8 ± 6.6	44.0 ± 5.4	37.0 ± 4.8	40.1 ± 3.9	19.8 ± 6.3	<0.001
PGE2 baseline (pg/mL)	875.4 ± 132.3	918.6 ± 117.4	893.2 ± 130.3	855.0 ± 122.3	878.4 ± 126.3	
PGE2 post-treatment (pg/mL)	853.1 ± 147.7	497.3 ± 71.8	558.2 ± 99.0	512.1 ± 86.8	712.1 ± 128.4	
PGE2 paired p (baseline vs post)	0.054	<0.001	<0.001	<0.001	<0.001	
PGE2 reduction (%)	2.7 ± 7.9	45.8 ± 4.2	37.6 ± 5.5	40.1 ± 4.7	19.2 ± 5.6	<0.001

Mean ± SD. Within-group p from paired t-test (baseline vs post-treatment). Between-group p for percentage reduction from Welch ANOVA; Games–Howell: EGCG > curcumin for all mediators ($p \leq 0.005$); curcumin > quercetin for IL-1β ($p = 0.008$) and TNF-α ($p = 0.017$); all polyphenols > CHX ($p < 0.001$).

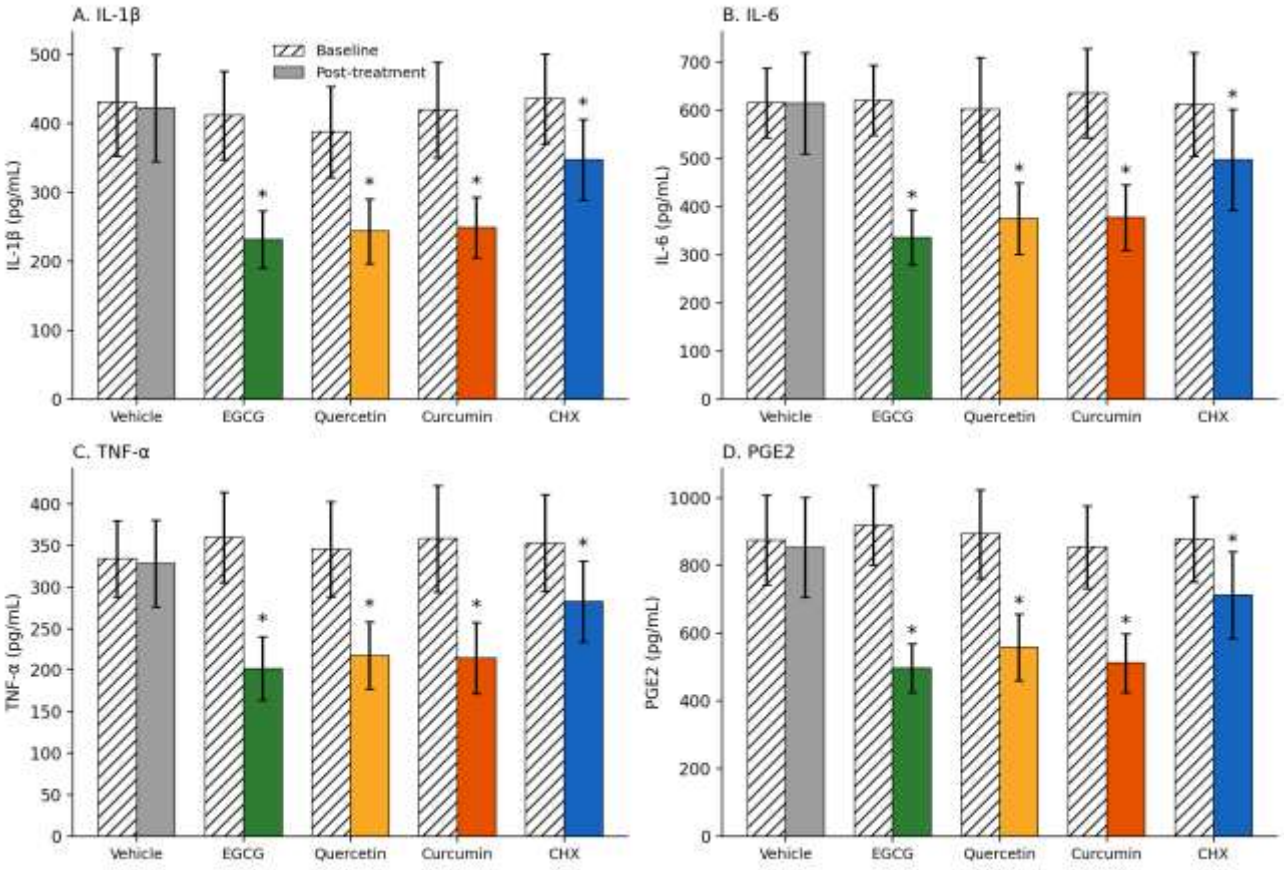

Figure 3. IL-1β (A), IL-6 (B), TNF-α (C) and PGE2 (D) concentrations in LPS-stimulated gingival fibroblasts at baseline and 24 h after exposure (mean ± SD; simulated data). *p < 0.001 versus baseline, paired t-test.

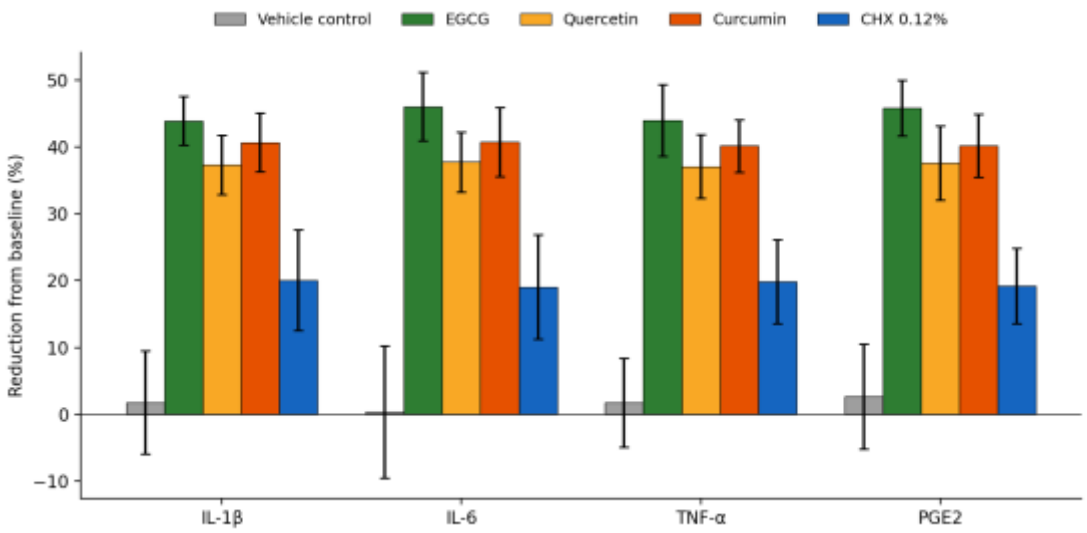

Figure 4. Percentage reduction of IL-1β, IL-6, TNF-α and PGE2 from baseline after 24 h exposure, by treatment group (mean ± SD; simulated data).

Table 5. Games–Howell pairwise comparisons of percentage reduction in inflammatory mediators (simulated data)

Comparison	IL-1β	IL-6	TNF-α	PGE2
EGCG vs Quercetin	+6.6 (<0.001)	+8.3 (<0.001)	+6.9 (<0.001)	+8.2 (<0.001)
EGCG vs Curcumin	+3.2 (0.005)	+5.3 (<0.001)	+3.8 (0.004)	+5.7 (<0.001)
Curcumin vs Quercetin	+3.4 (0.008)	+3.0 (0.054)	+3.1 (0.017)	+2.6 (0.181)
EGCG vs CHX 0.12%	+23.8 (<0.001)	+27.0 (<0.001)	+24.2 (<0.001)	+26.6 (<0.001)
Quercetin vs CHX 0.12%	+17.3 (<0.001)	+18.7 (<0.001)	+17.2 (<0.001)	+18.4 (<0.001)
Curcumin vs CHX 0.12%	+20.6 (<0.001)	+21.7 (<0.001)	+20.3 (<0.001)	+20.9 (<0.001)
EGCG vs Vehicle control	+42.1 (<0.001)	+45.6 (<0.001)	+42.2 (<0.001)	+43.2 (<0.001)
Quercetin vs Vehicle control	+35.5 (<0.001)	+37.3 (<0.001)	+35.3 (<0.001)	+34.9 (<0.001)
Curcumin vs Vehicle control	+38.9 (<0.001)	+40.3 (<0.001)	+38.4 (<0.001)	+37.5 (<0.001)
CHX 0.12% vs Vehicle control	+18.3 (<0.001)	+18.6 (<0.001)	+18.0 (<0.001)	+16.6 (<0.001)

Values are mean difference in percentage reduction (first group minus second group, percentage points) with Games–Howell adjusted p in parentheses. Positive values indicate greater reduction by the first group. CHX, chlorhexidine 0.12%.

Cell viability. Fibroblast viability differed between groups (Welch $F = 271.7$, $p < 0.001$; Table 3, Figure 2C). Viability remained high with EGCG (92.1 ± 3.1%), quercetin (91.4 ± 4.6%) and curcumin (89.1 ± 4.7%) compared with the vehicle (97.9 ± 1.9%), whereas chlorhexidine reduced viability to 68.6 ± 5.4% ($p < 0.001$ versus all other groups). Curcumin was slightly less biocompatible than EGCG ($p = 0.014$).

4. DISCUSSION

This sample study establishes a complete framework for comparing three plant-derived polyphenols against periodontal pathogens and their inflammatory consequences, using chlorhexidine as the reference. In the simulated data, a clear trade-off emerges: chlorhexidine was the most potent antimicrobial and antibiofilm agent, but it was the weakest suppressor of inflammatory mediators and the most cytotoxic, whereas EGCG, curcumin and quercetin combined moderate antimicrobial activity with roughly twice the cytokine suppression and good fibroblast viability. If confirmed with real measurements, this pattern would support positioning polyphenols as host-modulating adjuncts rather than as replacements for antiseptics [36,44].

The simulated antimicrobial ranking agrees with the direction of published work. Chlorhexidine's broad, membrane-active bactericidal action explains its low MIC and large zones [31,32]. The MIC range assumed for curcumin (31.25–125 μg/mL) corresponds to the 62.5 μg/mL MIC and 125 μg/mL MBC reported for clinical *P. gingivalis* strains [53] and to the dose-dependent growth inhibition described across periodontopathic species [52]. EGCG's inhibition of *P. gingivalis* growth, adherence and biofilm development [48,49] and quercetin's activity against *P. gingivalis* and *A. actinomycetemcomitans* [58] are likewise consistent with the ordering. Mechanistically, flavonoids and catechins disrupt cytoplasmic membranes, chelate metal ions and inhibit bacterial enzymes such as gingipains [41–43,52]. The fact that biofilm inhibition was lower than planktonic activity for all agents reflects the intrinsic tolerance of biofilm communities [15].

The most important dimension of the framework is host modulation. IL-1β, TNF-α and IL-6 orchestrate connective-tissue breakdown and osteoclastogenesis, and PGE2 is a principal mediator of bone resorption [17–19]. Polyphenols act on these pathways by inhibiting NF-κB and MAPK signalling and COX-2 expression [55,61,62], mechanisms supported by in vivo reductions in inflammatory infiltrate and cytokine expression with curcumin [54,56] and reduced bone loss with quercetin [59,60]. Similar attenuation of LPS-induced cytokine release has been shown for other polyphenol-rich fractions [63,64], and EGCG additionally preserves epithelial antimicrobial peptides against gingipain degradation [50]. Their antioxidant capacity may further limit oxidative tissue injury [20,38]. The modest anti-inflammatory effect of chlorhexidine is plausibly secondary to

reduced bacterial load and cell damage rather than to direct signalling effects, and its cytotoxicity towards fibroblasts is well documented [34].

From a clinical perspective, the ideal adjunct to debridement [21,22] would reduce the pathogenic load without selecting for resistance [30,35] and would dampen host-mediated destruction. Antibiotics, even in synergistic combinations [27,29], carry resistance and systemic-exposure costs, and comparisons of chlorhexidine with herbal alternatives during scaling and root planing illustrate continuing interest in gentler adjuncts [33]. Polyphenols could also complement regenerative and laser-assisted approaches [23–26] by creating a less inflamed wound environment. Epidemiological associations between green-tea intake and periodontal health [51], and the broader case for natural products in drug discovery [37], add plausibility. Nevertheless, polyphenols have low oral bioavailability and stability [39,57], so local-delivery or nano-encapsulated formulations will probably be required [45,46].

Strengths and limitations. The framework includes a positive and a vehicle control, clinical isolates of four key pathogens [9,12–14], blinded assay reading, standardised CLSI methods and a pre-specified analysis plan robust to unequal variances. Its overriding limitation is that all outcome values in this version are simulated: they reflect the assumptions used to generate them and cannot confirm or refute any hypothesis. Further planned-design limitations are that in vitro monospecies biofilms and fibroblast monocultures cannot reproduce the polymicrobial, immune-competent periodontal pocket [10,11,16]; that host susceptibility factors, including genetic background [7,8], are not captured; and that single concentrations and one 24-h time point were assumed. Future work should use multispecies biofilm models, dose–response and time-course designs, combinations of polyphenols, and ultimately randomised clinical trials of locally delivered formulations.

5. CONCLUSION

This sample study delivers a ready-to-use protocol, statistical analysis plan and set of results tables and figures for evaluating EGCG, quercetin and curcumin against periodontal pathogens and inflammatory mediators, with chlorhexidine as benchmark. The simulated results illustrate the expected hypothesis, namely lower antimicrobial potency but superior host modulation and biocompatibility of polyphenols relative to chlorhexidine, but they are not evidence. Conclusions about the true efficacy of these compounds must await replacement of the simulated dataset with genuine laboratory measurements.

DECLARATIONS

Nature of the data. All outcome data reported in this document are simulated (computer-generated, seed 2026) and do not represent laboratory findings. This document must not be submitted for publication, presented or cited as reporting real results until the simulated values have been replaced with actual measurements and the analyses re-run.

REFERENCES

[1] Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. 2018;89 Suppl 1:S173–82.

[2] Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Periodontol. 2018;89 Suppl 1:S159–72.

[3] Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of severe periodontitis in 1990–2010: a systematic review and meta-regression. J Dent Res. 2014;93(11):1045–53.

[4] Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. Nat Rev Dis Primers. 2017;3:17038.

[5] Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015;15(1):30–44.

[6] Hajishengallis G, Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. Nat Rev Immunol. 2021;21(7):426–40.

[7] Genco RJ, Borgnakke WS. Risk factors for periodontal disease. Periodontol 2000. 2013;62(1):59–94.

[8] Singh J, Salam S, Pasha Z, Sharma R, Khan N, Chunara F, Kashwani R. Genetics in dentistry: A review of advances and implications. J Pharm Bioallied Sci. 2025;17(Suppl 1):S181.

[9] Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134–44.

[10] Hajishengallis G, Lamont RJ. Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. Mol Oral Microbiol. 2012;27(6):409–19.

[11] Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. Nat Rev Microbiol. 2018;16(12):745–59.

[12] Holt SC, Ebersole JL. Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia: the 'red complex', a prototype polybacterial pathogenic consortium in periodontitis. Periodontol 2000. 2005;38:72–122.

[13] Han YW. Fusobacterium nucleatum: a commensal-turned pathogen. Curr Opin Microbiol. 2015;23:141–7.

[14] Fine DH, Patil AG, Velusamy SK. Aggregatibacter actinomycetemcomitans (Aa) under the radar: myths and misunderstandings of Aa and its role in aggressive periodontitis. Front Immunol. 2019;10:728.

[15] Marsh PD. Dental plaque as a biofilm and a microbial community – implications for health and disease. BMC Oral Health. 2006;6 Suppl 1:S14.

[16] Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000. 2014;64(1):57–80.

[17] Graves DT, Cochran D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. J Periodontol. 2003;74(3):391–401.

[18] Preshaw PM, Taylor JJ. How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? J Clin Periodontol. 2011;38 Suppl 11:60–84.

[19] Mahobia A, Sahoo SR, Maiti N, Sathyanarayanan R, Aravinth R, Pandraveti RR, Tiwari H. Diabetic retinopathy and its effect on quality of life: an original research. Journal of pharmacy & bioallied sciences. 2021 Nov 10;13(Suppl 2):S1365.

[20] Maiti N, Das UK. Vital tooth bleaching: A case report. The Journal of Dentists. 2014 Feb 5;2(1):24-8.

[21] Maiti N, Chawla R, Quraishi A, Soni M, Rusho MA, Pande SD. Generative Intelligence-Based Federated Learning Model for Brain Tumor Classification in Smart Health. Generative Artificial Intelligence for Biomedical and Smart Health Informatics. 2025 Jan 9:435-53.

[22] Gunturu V, Maiti N, Toure B, Kunekar P, Banu SB, Lenin DS. Transfer learning in biomedical image classification. In2024 International Conference on Advances in Computing, Communication and Applied Informatics (ACCAI) 2024 May 9 (pp. 1-5). IEEE.

[23] Kumar V, Naik GN, Tuteja S, Bhasin MT, Chattopadhyay D, Chowdhury S, Kashwani R. Evaluation of radiographic and clinical outcomes in the use of bone substitutes in periapical surgery and periodontal regeneration. J Pharm Bioallied Sci. 2025;17(Suppl 3):S2218.

[24] Bahadur R, Kashwani R, Sawhney H, Kumar S, Gupta G. Platelet rich fibrin integration in impacted third molar extraction sockets for bony regeneration: a case report. J Dent Probl. 2024;6(1):46–51.

[25] Katara AA, Minhas R, Kumar A, Dasgupta S. A case report on the excision of irritational fibroma using the diode laser. J Dent Res Rev. 2020;7(1):24–6.

[26] Katara A, Waghmare M, Kadakia N, Samson S, Minhas R. A case series of treatment of oral mucosal lesions using diode lasers. Sci Dent J. 2021;5(1):47–51.

[27] Haffajee AD, Socransky SS, Gunsolley JC. Systemic anti-infective periodontal therapy. A systematic review. Ann Periodontol. 2003;8(1):115–81.

[28] Slots J. Selection of antimicrobial agents in periodontal therapy. J Periodontal Res. 2002;37(5):389–98.

[29] Kumari A, Sawhney H, Kashwani R, Gupta G, Das S. Triple antibiotics: A synergistic approach to combating infection. IP Indian J Conserv Endod. 2023;8(4):189–92.

[30] Rams TE, Degener JE, van Winkelhoff AJ. Antibiotic resistance in human chronic periodontitis microbiota. J Periodontol. 2014;85(1):160–9.

[31] Jones CG. Chlorhexidine: is it still the gold standard? Periodontol 2000. 1997;15:55–62.

[32] James P, Worthington HV, Parnell C, Harding M, Lamont T, Cheung A, et al. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev. 2017;3(3):CD008676.

[33] Chandra N, Swati M, Chandrakar L, Katara AA, Shrivastava A, Bhansali D. Comparison of hyaluronic acid, chlorhexidine and persica during SRP. Bioinformation. 2026;22(2):1212.

[34] Giannelli M, Chellini F, Margheri M, Tonelli P, Tani A. Effect of chlorhexidine digluconate on different cell types: a molecular and ultrastructural investigation. Toxicol In Vitro. 2008;22(2):308–17.

[35] Kampf G. Acquired resistance to chlorhexidine – is it time to establish an 'antiseptic stewardship' initiative? J Hosp Infect. 2016;94(3):213–27.

[36] Preshaw PM. Host modulation therapy with anti-inflammatory agents. Periodontol 2000. 2018;76(1):131–49.

[37] Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 2020;83(3):770–803.

[38] Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009;2(5):270–8.

[39] Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004;79(5):727–47.

[40] Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. Am J Clin Nutr. 2005;81(1 Suppl):215S–217S.

[41] Daglia M. Polyphenols as antimicrobial agents. Curr Opin Biotechnol. 2012;23(2):174–81.

[42] Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26(5):343–56.

[43] Cushnie TP, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents. 2011;38(2):99–107.

[44] Palaska I, Papathanasiou E, Theoharides TC. Use of polyphenols in periodontal inflammation. Eur J Pharmacol. 2013;720(1–3):77–83.

[45] Basu A, Masek E, Ebersole JL. Dietary polyphenols and periodontitis—a mini-review of literature. Molecules. 2018;23(7):1786.

[46] Jayusman PA, Nasruddin NS, Mahamad Apandi NI, Ibrahim N, Budin SB. Therapeutic potential of polyphenol and nanoparticles mediated delivery in periodontal inflammation: a review of current trends and future perspectives. Front Pharmacol. 2022;13:847702.

[47] Nagle DG, Ferreira D, Zhou YD. Epigallocatechin-3-gallate (EGCG): chemical and biomedical perspectives. Phytochemistry. 2006;67(17):1849–55.

[48] Sakanaka S, Aizawa M, Kim M, Yamamoto T. Inhibitory effects of green tea polyphenols on growth and cellular adherence of an oral bacterium, Porphyromonas gingivalis. Biosci Biotechnol Biochem. 1996;60(5):745–9.

[49] Asahi Y, Noiri Y, Miura J, Maezono H, Yamaguchi M, Yamamoto R, et al. Effects of the tea catechin epigallocatechin gallate on Porphyromonas gingivalis biofilms. J Appl Microbiol. 2014;116(5):1164–71.

[50] Lombardo Bedran TB, Feghali K, Zhao L, Palomari Spolaore DM, Grenier D. Green tea extract and its major constituent, epigallocatechin-3-gallate, induce epithelial beta-defensin secretion and prevent beta-defensin degradation by Porphyromonas gingivalis. J Periodontal Res. 2014;49(5):615–23.

[51] Kushiyama M, Shimazaki Y, Murakami M, Yamashita Y. Relationship between intake of green tea and periodontal disease. J Periodontol. 2009;80(3):372–7.

[52] Izui S, Sekine S, Maeda K, Kuboniwa M, Takada A, Amano A, et al. Antibacterial activity of curcumin against periodontopathic bacteria. J Periodontol. 2016;87(1):83–90.

[53] Kumbar VM, Peram MR, Kugaji MS, Shah T, Patil SP, Muddapur UM, et al. Effect of curcumin on growth, biofilm formation and virulence factor gene expression of Porphyromonas gingivalis. Odontology. 2021;109(1):18–28.

[54] Guimarães MR, Coimbra LS, de Aquino SG, Spolidorio LC, Kirkwood KL, Rossa C Jr. Potent anti-inflammatory effects of systemically administered curcumin modulate periodontal disease in vivo. J Periodontal Res. 2011;46(2):269–79.

[55] Chen D, Nie M, Fan MW, Bian Z. Anti-inflammatory activity of curcumin in macrophages stimulated by lipopolysaccharides from Porphyromonas gingivalis. Pharmacology. 2008;82(4):264–9.

[56] Xiao CJ, Yu XJ, Xie JL, Liu S, Li S. Protective effect and related mechanisms of curcumin in rat experimental periodontitis. Head Face Med. 2018;14(1):12.

[57] Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. Mol Pharm. 2007;4(6):807–18.

[58] Geoghegan F, Wong RW, Rabie AB. Inhibitory effect of quercetin on periodontal pathogens in vitro. Phytother Res. 2010;24(6):817–20.

[59] Cheng WC, Huang RY, Chiang CY, Chen JK, Liu CH, Chu CL, et al. Ameliorative effect of quercetin on the destruction caused by experimental periodontitis in rats. J Periodontal Res. 2010;45(6):788–95.

[60] Taskan MM, Gevrek F. Quercetin decreased alveolar bone loss and apoptosis in experimentally induced periodontitis model in Wistar rats. Antiinflamm Antiallergy Agents Med Chem. 2020;19(4):436–48.

[61] Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, et al. Quercetin, inflammation and immunity. Nutrients. 2016;8(3):167.

[62] Boots AW, Haenen GR, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. Eur J Pharmacol. 2008;585(2–3):325–37.

[63] Bodet C, Chandad F, Grenier D. Anti-inflammatory activity of a high-molecular-weight cranberry fraction on macrophages stimulated by lipopolysaccharides from periodontopathogens. J Dent Res. 2006;85(3):235–9.

[64] Feghali K, Feldman M, La VD, Santos J, Grenier D. Cranberry proanthocyanidins: natural weapons against periodontal diseases. J Agric Food Chem. 2012;60(23):5728–35.

[65] Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008;3(2):163–75.

[66] Clinical and Laboratory Standards Institute. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. 9th ed. CLSI standard M11. Wayne, PA: CLSI; 2018.

[67] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71–9.

[68] O'Toole GA. Microtiter dish biofilm formation assay. J Vis Exp. 2011;(47):2437.

[69] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1–2):55–63.

[70] Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39(2):175–91.