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Antimicrobial Photodynamic Therapy With Curcumin And Rose Bengal Against Oral Biofilms: Light-Dependent And Independent Effects

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Abstract

Aim: *Streptococcus mutans and Candida albicans serve as crucial microorganisms in oral illnesses, associated with tooth caries and opportunistic infections. Their biofilm-forming ability reduces susceptibility to conventional antimicrobials, complicating treatment.(1,2) This study aimed to evaluate the antimicrobial efficacy of curcumin (CUR) and Rose Bengal (RB) in photodynamic therapy (aPDT) against mono-species biofilms of Streptococcus mutans and Candida albicans, comparing light-dependent and light-independent effects.*

Methods And Methods: *Clinical isolates of S. mutans (n=100) and C. albicans (n=100) from patients (Jan–Sep 2024) were treated with optimized concentrations of curcumin (CUR, 100 μM) or Rose Bengal (RB, 50 μM), determined via pilot testing. Biofilms were exposed to blue light (450–470 nm, 1000 mW/cm², 1 min) or kept dark. Metabolic activity was assessed via resazurin assay. Statistics: ANOVA/Tukey's (C. albicans) and Kruskal-Wallis/Mann-Whitney (S. mutans; $p^* < 0.05$).*

Results: *Light-activated CUR and RB significantly reduced biofilm viability versus controls ($p^* < 0.05$). C. albicans were more susceptible than S. mutans, with RB showing superior antifungal activity ($p^* < 0.05$). CUR exhibited light-independent effects.*

Conclusion: *aPDT with CUR/RB effectively disrupts oral biofilms, demonstrating dual light-dependent and independent mechanisms. This supports its use as an adjunct for caries and candidiasis management.*

Keywords: *Curcumin, Laser Therapy, Photochemotherapy, Rose Bengal, Biofilms*

INTRODUCTION:

Dental caries persist as significant worldwide health issues, with *Streptococcus mutans* and *Candida albicans* recognized as primary sources owing to their ability to form biofilms and exhibit antibiotic resistance.(3,4)*Streptococcus mutans biofilms facilitate dental caries via acid formation and the formation of extracellular polysaccharides.*(3) *C. albicans* causes opportunistic infections, particularly in immuno-compromised individuals. Traditional therapies, like chlorhexidine and antifungals, encounter restrictions due to microbe resistance, biofilm durability, and host toxicity(5,6)

Antimicrobial photodynamic treatment (aPDT) is now recognized as a potential approach, utilizing light-activated photosensitive substances like curcumin and Rose Bengal to produce ROS, or reactive oxygen species, that compromise microbiological survival. (7) New studies underscore the efficiency of aPDT against mono-species biofilms; nonetheless, substantial shortcomings impede its practical application. Comparative research on natural (curcumin) versus synthetic (Rose Bengal) photosensitizers are limited, especially concerning oral infections.(8) Secondly, the inconsistency regarding light parameters (e.g., wavelength, irradiance) among research hinders the uniformity of protocols. (9) The light-independent antibacterial properties of photosensitizers, including curcumin's capacity to impede the detection of quorum, are inadequately defined in biofilm studies.(10) Recognizing these weaknesses is crucial to enhance aPDT for practical dentistry uses.

This research addresses existing information shortages by methodically assessing curcumin and Rose Bengal against mono-species biofilms of *S. mutans* and *C. albicans* using controlled blue light (450–470 nm). We measure decreases in biofilm viability following aPDT while assessing antibacterial effects that rely on light vs those that are independent of light.(11) Our research seeks to develop evidence-based procedures for aPDT in the therapy of caries,

providing a minimally invasive, resistance-free complement to existing treatments. This study enhances the therapeutic applicability of aPDT in dentistry by clarifying molecular distinctions among these photosensitizers.(12)

Materials and Methods:

Curcumin (CUR) and Rose Bengal (RB) were prepared as stock solutions for antimicrobial photodynamic therapy (aPDT). CUR (50 mg/mL) was dissolved in dimethyl sulfoxide (DMSO) due to its restricted solubility in water and afterwards sterilized via 0.22 μm filtration. RB (50 mg/mL) was dissolved in sterile distilled water and then filtered. Working concentrations were diluted to 136 μM (CUR, in a 1:1 PBS/DMSO solution) and 0.1 μM (RB, in PBS), protected from light to maintain stability.(13,14)

Working concentrations of both Curcumin and Rose bengal were selected based on established efficacy ranges reported in previous aPDT studies. (15,16)

Isolation of Candida albicans:

Oral swabs from 100 patients were inoculated onto Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 24 to 48 hours. Creamy colonies were microscopically identified as *C. albicans* (oval yeast/pseudohyphae).(17)

Isolation and Identification of *Streptococcus mutans*:

Clinical specimens were procured from 100 patients (aged 25–60 years) with profound carious lesions at the University of Al-Kufa (January–September 2024). Swab samples were cultivated aerobically at 37°C for 24 hours and identified with the VITEK-2 system (BioMérieux). Biofilm-producing bacteria were identified using ELISA (optical density >0.240 at 570 nm).(18)

Biofilm Formation Assay:

Biofilms of S. mutans (cultured in Brain Heart Infusion-glucose) and *C. albicans* (cultured in Sabouraud Dextrose-glucose) were developed in 96-well plates at 37°C for 24 hours. Planktonic cells were eliminated using PBS washing, and biofilms were preserved using 33% glacial acetic acid. Biomass was measured by optical density (630 nm) utilizing an ELISA reader(19)]A number of samples of 10 isolates per group was chosen to guarantee statistical power while preserving experimental feasibility. The isolates were methodically categorized into separate treatment and control groups to evaluate both light-dependent and light-independent effects. (20)

For Streptococcus mutans:

Group I as –antibacterial activity of *Curcumin against S. mutans* with light activation.
Group I bs –antibacterial activity of *Curcumin against S. mutans* without light activation.
Group II as –antibacterial activity of *Rose Bengal against S. mutans* with light activation.
Group II bs –antibacterial activity of *Rose Bengal against S. mutans* without light activation.
Group III as – antibacterial activity of light activation *against S. mutans* (no photosensitizer, only light activation).
Group III bs – *S. mutans* only (no photosensitizer, no light activation).

For Candida albicans:

Group I ac –antifungal activity of *Curcumin against C. albicans* with light activation.
Group I bc – antifungal activity of *Curcumin against C. albicans* without light activation.
Group II ac – antifungal activity of *Rose Bengal against C. albicans* with light activation.
Group II bc – antifungal activity of *Rose Bengal against C. albicans* without light activation.
Group III ac – antifungal activity of light activation *against C. albicans* (no photosensitizer, only light activation).
Group III bc– *C. albicans* only (no photosensitizer, no light activation).

aPDT Parameters and Light Activation:

Photodynamic treatment was conducted with a blue LED (450-470 nm) at an intensity of 1000 mW/cm² for 1 minute (60 J/cm² dosage), with the light source placed 2 cm above the samples. Photosensitizers were incubated for five minutes prior to irradiation, followed by a 60-minute incubation period post-exposure. Temperature was observed with a variation of less than 2°C during illumination. Control groups comprised samples exposed solely to light and those that were untreated. All procedures adhered to defined norms (21), with samples protected from ambient light to prevent unintended activation.(22)

Biofilm Inhibition Assay (96-Well Plate Method)

The efficacy of curcumin and rose bengal in inhibiting biofilm formation was assessed using a standardized 96-well plate test. Bacterial suspensions were calibrated to the 0.5 McFarland norm (~ 1.5×10^8 CFU/mL) in BHI-glucose broth. Aliquots of 200 µL were dispensed into wells (three copies per isolate) and incubated at 37°C for 24 hours. Following incubation, planktonic cells were eliminated using gentle washing with PBS, and adhering biofilms were fixed using 33% glacial acetic acid (200 µL/well). The biomass of the biofilm was assessed by assessing optical density (OD_{630}nm) with an ELISA reader. The biofilm inhibition percentage was computed as follows:

Biofilm Inhibition (%) = [(ODcontrol − ODtreatment) / ODcontrol] × 100

where:

- ODcontrol = Optical density of untreated biofilm (positive control)
- ODtreatment = Optical density of extract-treated biofilm

The assay followed protocols by Bostanghadiri et al. (2021), with modifications for photosensitizer testing.

Experimental Design

The research utilized separate experimental groups for every microbe. For S. mutans (100 µM treatment), Group I as was administered CUR with light activation, Group I bs received CUR without light, Group II as was given RB with light activation, and Group II bs received RB without light. Control groups comprised light-only exposure (III as) and untreated samples (III bs). The same group structure was preserved for *C. albicans* (I ac , I bc , II ac , II bc) testing, utilizing the optimal concentration of 50 µM. This arrangement facilitated a thorough evaluation of these two photosensitizers during light-activated and non-activated conditions with respect to each microbiological focus. Control groups were incorporated to mitigate potential confounding variables. Group III (as) comprised S. mutans subjected just to light (without a photosensitizer), guaranteeing that any effects detected were not attributable to light-induced alterations. Group III (bs) functioned as an untreated control, establishing a baseline for biofilm survival under regular settings. This systematic strategy facilitated an obvious assessment of the antimicrobial properties of CUR and RB, both with and without light activation, while reducing bias and assuring dependable data. Same control groups were done for candida sample.

Data Statement: Data will be available upon request.

Statistical Analysis: Data were analyzed using SPSS v26 (IBM). Normality was assessed via Shapiro-Wilk test. For C. albicans, ANOVA with Tukey's post hoc test was applied; for *S. mutans,* non-parametric Kruskal-Wallis and Mann-Whitney tests were used. Significance was set at $p^* < 0.05$.

Normality Tests:

The Shapiro-Wilk test confirmed normal distribution for all *Candida albicans* groups ($p^* > 0.05$). For *Streptococcus mutans*, Groups I as, I bs , II bs, and III bs exhibited normality ($p^* > 0.05$), while Groups II as and III as were non-normally distributed ($p^* < 0.05$). (Table 1, Table 2)

Table1 Normality of Candida albicans groups using Kolmogorov-Smirnov and Shapiro-Wilk test.

groups	Shapiro-Wilk		
	Statistic	df	Sig.
group I ac	.896	10	.200
group III ac	.949	10	.660
group I bc	.947	10	.628
group II bc	.916	10	.325
group III bc	.909	10	.275

Table2 Normality of Streptococcus mutans groups using Kolmogorov-Smirnov and Shapiro-Wilk test.

groups	Shapiro-Wilk		
	Statistic	df	Sig.
group I as	.945	10	.604

group II as	.734	10	.002
group III as	.924	10	.390
group I bs	.939	10	.542
group II bs	.954	10	.712
group III bs	.740	10	.003

Descriptive Statistics:

- *Candida albicans:* The study's results indicate that group rose Bengal with light activation (1.0±0.0) exhibits the highest mean value, whilst group light activation only (0.023±0.1889) demonstrates the lowest mean value for the Biofilm inhibition ratio (%) of Candida albicans groups. (Table 3, Figure 2)
- *Streptococcus mutans*: The study's results indicate that group Rose Bengal with light activation, with a mean value of (0.9440±0.08003), exhibits the highest mean, whilst group light activation only, with a mean value of (0.008±0.01135), demonstrates the lowest mean for the Biofilm inhibition ratio (%) of the Streptococcus mutans groups. (Table 4, Figure 3)

Table3 Mean, standard deviation, Mean Rank, Minimum and Maximum of the Candida albicans groups.

groups	Mean	Std. Deviation	Std. Error	Minimum	Maximum
group I ac	.8070	.06395	.02022	.72	.89
group II ac	1.0000	.00000	.00000	1.00	1.00
group III ac	.2160	.06802	.02151	.13	.33
group I bc	.5500	.10562	.03340	.40	.70
group II bc	.7620	.09402	.02973	.56	.87
group III bc	.0230	.01889	.00597	.00	.05
Total	.5597	.35136	.04536	.00	1.00

Table 4 Mean, standard deviation, Mean Rank, Minimum, Maximum and Kruskal-Wallis Test of the Streptococcus mutans groups.

Group	**Mean**	**Std. Deviation**	**Mean Rank**	**Minimum**	**Maximum**	**p-value**
Group I as	0.633	0.11324	44.40	0.43	0.77	.000
Group II as	.9440	0.08003	55.50	0.8	1	
Group III as	.1450	0.03504	15.50	0.1	0.19	

Group I bs	.3050	0.06241	26	0.22	0.4	
Group II bs	.4620	0.07345	36.10	0.31	0.56	
Group III bs	0.008	0.01135	5.5	0	0.03	

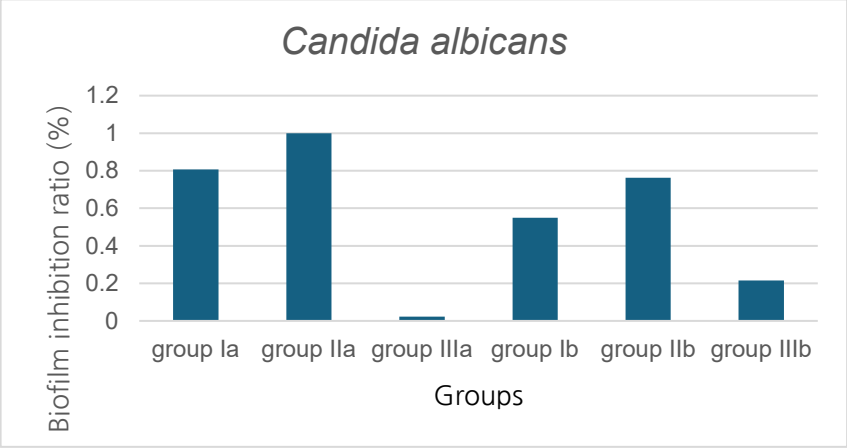

Figure 2 Bar chart illustrates mean value of the Biofilm inhibition ratio (%) of the Candida albicans groups

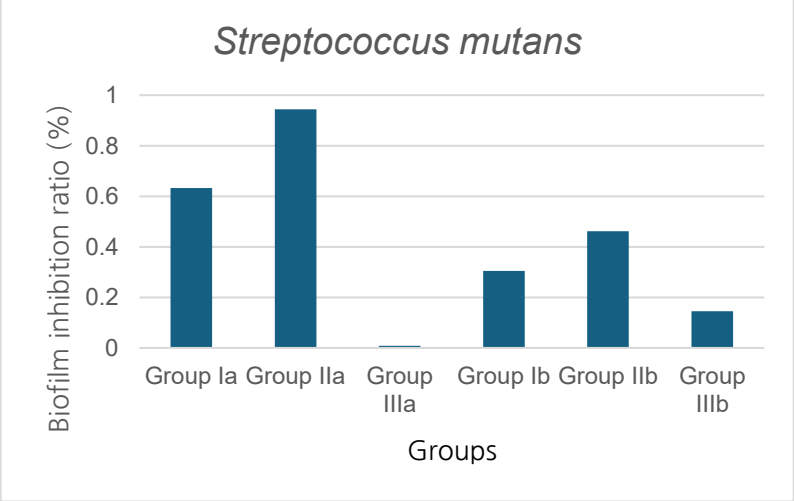

Figure 3 Bar chart illustrates mean value of the Biofilm inhibition ratio (%) of the Streptococcus mutans groups.

Inferential Analyses:

1. ANOVA (*Candida albicans*): A statistically significant difference was observed among groups (F = 289.884, $p < 0.001$). (Table 5)

The result of Tukey's post hoc test showed that the group Curcumin with light has a statistically significant difference less than Curcumin without light activation group and both negative and positive control groups. Also, Rose Bengal with light activation group has a statistically significant difference less than Curcumin without light activation group, negative and positive control groups. While the negative control group has a statistically significant difference more than the positive control group. Also, Curcumin without light activation group has a statistically significant difference more than Rose Bengal without light activation group and the Rose Bengal without light activation has a statistically significant difference less than curcumin without light activation groups and both control groups. (table 6)

2. The Kruskal-Wallis test indicates a statistically significant difference ($P < 0.05$) among the groups of Streptococcus mutans. (Table 4)

The result of Mann-Whitney U test showed that the group Curcumin with light has a statistically significant difference less than Curcumin without light activation group and both negative and positive control groups. Also, Rose Bengal with light activation group has a statistically significant difference less than Curcumin without light activation group, negative and positive control groups. While the negative control group has a statistically significant difference more than the positive control group. Also, Curcumin without light activation group has a statistically significant difference more than Rose Bengal without light activation group and the Rose Bengal without light activation has a statistically significant difference less than curcumin without light activation groups and both control groups. (Table 7)

Table 5 Testing one-way ANOVA of colony forming unit per millileter among groups of Candida albicans.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.022	5	1.404	289.884	.000
Within Groups	.262	54	.005		
Total	7.284	59			

Table 6 Tukey's post hoc test comparison between each two groups of Candida albicans.

(I) groups	(J) groups	Mean Difference (I-J)	Std. Error	Sig.
I ac	II ac	-.19300*	.03113	.000
I ac	III ac	.78400*	.03113	.000
I ac	I bc	.25700*	.03113	.000
I ac	II bc	.04500	.03113	.699
I ac	III bc	.59100*	.03113	.000
II ac	III ac	.97700*	.03113	.000
II ac	I bc	.45000*	.03113	.000
II ac	II bc	.23800*	.03113	.000
II ac	III bc	.78400*	.03113	.000
III ac	I bc	-.52700*	.03113	.000
III ac	II bc	-.73900*	.03113	.000
III ac	III bc	-.19300*	.03113	.000
I bc	II bc	-.21200*	.03113	.000
I bc	III bc	.33400*	.03113	.000
II bc	III bc	.54600*	.03113	.000

*.The mean difference is significant at the 0.05 level.

Table 7 Mann-Whitney U test comparison between each two groups of Streptococcus mutans groups.

Group (I)	Mean Rank	Group (j)	Mean Rank	P- value
Group I as	5.5	Group II as	15.5	0
Group I as	15.5	Group III bs	5.5	0
Group I as	15.5	Group I bs	5.5	0
Group I as	14.4	Group II bs	6.6	0.003
Group I as	15.5	Group III as	5.5	0
Group II as	15.5	Group III bs	5.5	0
Group II as	15.5	Group I bs	5.5	0
Group II as	15.5	Group II bs	5.5	0

Group II as	15.5	Group III as	5.5	0
Group III bs	5.5	Group I bs	15.5	0
Group III bs	5.5	Group II bs	15.5	0
Group III bs	5.5	Group III as	15.5	0
Group I bs	6	Group II bs	15	0.001
Group I bs	15.5	Group III as	5.5	0
Group II bs	15.5	Group III as	5.5	0

DISCUSSION:

The findings of this research indicate that antimicrobial photodynamic therapy utilizing curcumin and rose bengal can successfully impede biofilm formation of two principal oral pathogens, *Streptococcus mutans* and Candida albicans. Our findings enhance current developments in photodynamic treatment, offering new perspectives on dosage optimization and pathogen-specific reactions. The choice of 100 μM for *S. mutans* and 50 μM for *C. albicans* was determined through organized pilot testing of five concentrations, a methodology recently corroborated by Dovigo et al. (2013) in their study on photosensitizer improvement for oral biofilms. (23) The concentration-dependent efficacy was notably observed in the nearly total suppression of *C. albicans* biofilms by rose bengal at 50 μM, corroborating Dai et al.'s (2012) demonstration of RB's robust antifungal action at comparable concentrations.(24)

The divergent responses of the two infections necessitate further investigation. Rose bengal had more efficacy against C. albicans, whereas curcumin displayed broader effects against *S. mutans*, including significant light-independent antimicrobial properties. This discovery corroborates the expanding evidence indicating that curcumin exhibits many modes of action that go beyond photodynamic effects, include quorum sensing reduction and explicit membrane disruption, previously reported by Kauser et al. (2023).(25) The light-independent efficacy of curcumin against *S. mutans* biofilms (Group I bs: 0.305 ± 0.062) indicates possible therapeutic uses in scenarios where light activation is problematic. (26)

From a therapeutic standpoint, these findings hold significant implications for the management of two prevalent oral infections. The effectiveness of rose bengal against *C. albicans* at a concentration of 50 μM indicates its potential utility in treating oral candida infections in immunocompromised patients, as highlighted in recent clinical guidelines by Costa et al. (2023).(27) The synergistic light-dependent and independent actions of curcumin may provide a flexible strategy for avoiding the development of dental caries, applicable in several clinical contexts, ranging from clinical treatments to at-home care solutions. The recent systematic study by Pérez-Laguna et al. (2023) emphasized this dual activity, this underscored the necessity for adaptable therapeutic alternatives in caries management. (28)

From a therapeutic standpoint, these discoveries possess transformational implications for the treatment of oral diseases. The swift biofilm rupture induced by rose bengal has the potential to transform the management of recurring candidal infections, minimizing reliance on systemic antifungals and the associated adverse effectsCurcumin's dual techniques, both light-dependent and independent, provide exceptional adaptability for preventing the development of dental caries. This may manifest clinically as (1) light-activated antimicrobial photodynamic therapy for in-office caries management, (2) curcumin-enriched mouth rinses or varnishes for from home biofilm regulation, and (3) supplementary application with fluoride to augment preventive measures in high-risk individuals. This adaptability mitigates a significant deficiency in caries management, as biofilm persistence frequently compromises conventional therapy.(29) Curcumin's light-independent actions may be crucial in low-resource environments without laser or light devices, hence broadening access to sophisticated antibacterial methods. (30)

This study's experimental approach tackled various methodological issues recognized in prior studies. We employed standardised biofilm assays in 96-well plates, as advised by Bostanghadiri et al. (2021) (19), facilitated accurate measurement of therapy effects. The incorporation includes light-activated and non-light groups for a thorough evaluation of photosensitizer activity, surpassing the scope of the majority of prior investigations that predominantly

concentrated on light-dependent effects. This comprehensive study corresponds with recent literature advocating for an in-depth investigation into photosensitizer mechanisms, as described by Cieplik et al. (2019) in a review of aPDT procedures. (6,21)

Numerous considerations must be acknowledged while analyzing these findings. Although single-species biofilms offer standardized laboratory conditions, they may not accurately reflect the complicated microbial communities occurring in clinical environments. A recent study has highlighted the significance of creating polymicrobial biofilm systems that more accurately replicate oral ecosystem.(31) Moreover, although our in vitro findings are encouraging, their implementation in clinical environments necessitates thorough assessment of both safety and effectiveness in human trials, a difficulty highlighted in the previous in vivo research by Kim et al. (2024). (32) Further study should emphasize (1) clinical research that confirms the above results in vivo, (2) formulation studies to refine delivery methods (e.g., mucoadhesive gels for oral candidiasis), and (3) explorations of beneficial combinations with probiotics or nanoparticles to improve biofilm infiltration. (33,34)

These findings support aPDT as a stewardship tool to reduce antibiotic/antifungal use in oral infections, aligning with global AMR mitigation strategies. This work presents robust evidence supporting the application of curcumin and rose bengal in antimicrobial photodynamic treatment targeting two clinically relevant oral infections. The concentration-related effects and varied reactions across pathogens provide critical insights for formulating targeted treatment procedures. These findings significantly enhance the expanding corpus of research endorsing photodynamic treatment as an important asset in oral healthcare. The combination of demanding in vitro testing with clinically applicable concentrations and settings, as illustrated here, signifies a crucial advancement toward the evidence-based application of these potential therapeutic approaches.

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Ethical Statement: The study was approved by the University of Baghdad Ethics Committee (Ref. 949, 14 October 2024) in compliance with the Declaration of Helsinki.

Conflict of Interest: The authors declare no conflict of interest.

Declaration of Generative AI and AI-assisted technologies: Not applicable.

Author Contributions: MRH and ZNJ conceived the study. ZNJ performed experiments and analyzed data. ZNJ drafted the manuscript; MRH reviewed/edited. MRH supervised the project.

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