PATNA WOMEN'S COLLEGE AUTONOMOUS 1940

IRIS
Journal for Young Scientists
ISSN 2278 – 618X **(Print)**
ISSN 2278 – 6384 **(Online)**

http://www.patnawomenscollege.in/journal

Evaluation of Effectiveness of Blue, Red and UV Light for Decontamination of Toothbrushes against Common Pathogens

• **Sapna Kumari** • **Ayushi Rani** • **Shruti Solomon** • **Nirupama Soreng**

Received : October, 2024
Accepted : January, 2025
Corresponding Author : **Sapna Kumari**

Abstract: *Toothbrushes serve as critical oral hygiene tools but can also harbor and transmit microbial pathogens, posing a potential health risk. This study evaluated the antimicrobial efficacy of blue light, red light, and UV light as innovative decontamination methods for toothbrushes. The investigation focused on their ability to inactivate common oral and environmental pathogens, including Staphylococcus aureus, Escherichia coli, and Candida albicans. Toothbrushes contaminated with these pathogens were exposed to specific wavelengths and durations of each light type. Results demonstrated significant variations in microbial reduction rates, with UV light achieving the highest decontamination efficiency, followed by blue light, while red light showed moderate effects. These findings highlight the potential of non-invasive, light-based technologies in maintaining toothbrush hygiene, offering a safe and effective alternative to chemical disinfectants. Further research is recommended to optimize exposure conditions and assess long-term efficacy under real-world usage scenarios.*

Keywords: *Antimicrobial properties, Blue light, Red light, UV light, Toothbrush hygiene, Phototherapy, Photosensitizers.*

Sapna Kumari
Assistant Professor, Department of Zoology,
Patna Women's College (Autonomous),
Bailey Road, Patna–800 001, Bihar, India
E-mail : sapna.zoo@patnawomenscollege.in

Ayushi Rani
B.Sc. III year, Zoology (Hons.), Session : 2022-2025,
Patna Women's College (Autonomous),
Patna University, Patna, Bihar, India

Shruti Solomon
B.Sc. III year, Zoology (Hons.), Session : 2022-2025,
Patna Women's College (Autonomous),
Patna University, Patna, Bihar, India

Nirupama Soreng
B.Sc. III year, Zoology (Hons.), Session : 2022-2025,
Patna Women's College (Autonomous),
Patna University, Patna, Bihar, India

Introduction:

Oral health is an essential part of systemic health and the overall well-being of an individual (Karibasappa et al. 2011). Oral microbiome consists of various bacteria, viruses, and fungi that are responsible for causing a number of oral diseases (Paster et al. 2001). The oral cavity contains more than 700 types of bacteria and has the ideal temperature and humidity level for bacterial growth. It is, therefore, necessary to periodically remove bacteria from the teeth or tongue in the mouth, and one of the most common and effective methods for this is tooth brushing (Beneduce et al., 2010). Toothbrushes may become heavily contaminated with microorganisms from the oral cavity, environment, hands, aerosol contamination, and storage containers (Gujjari et al., 2011). Toothbrushes are likely to be stored

in bathrooms where it can harbor species such as *Streptococcus mutans*, *Lactobacillus*, and *Candida albicans*, which have been found in the oral cavity. The wet environment in the bathroom, where brushes are usually kept, may facilitate the bacterial growth and the cross contamination, especially that one which happens through aerosols produced during the water passage in lavatories, with enteric types and *Pseudomona*s from the toilets and sanitary drainage (Dhifaf, 2011). Potentially pathogenic bacteria, such as *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas,* and viruses such as herpes simplex virus can also be found. If a contaminated toothbrush is used repeatedly inside the oral cavity, it can cause oral and even systemic infection (Sato et al. 2004). Elderly people with a weakened immune system who are exposed to *Staphylococcus aureus* "superbug" strains can develop sepsis and enteritis. Importantly, *Staphylococcus aureus* generates exotoxins known as enterotoxins, which cause food poisoning, resulting in symptoms of nausea, vomiting, abdominal pain, and diarrhea (Park et al., 2002; Lee et al., 2008; Lee et al., 2014). The tooth brush may get contaminated by *Streptococcus*, *Staphylococcus* and *Lactobacilli* (Fernandez and Cesar, 2006). These bacteria are implicated in the causation of many life-threatening diseases such as infective endocarditis besides influencing the occurrence of oral diseases such as dental caries and gingivitis (Boylan et al., 2008). The increasing number of antibiotic resistant strains of microorganisms makes it even more important to develop antibiotic-free alternative treatments for decontamination of toothbrushes (Bush et al., 2011). Photodynamic therapy, has shown great potential against numerous pathogens and uses light of specific wavelength to stimulate an exogenously supplied photosensitizer, eliciting formation of toxic levels of reactive oxygen intermediates (Wainwright et al., 1998). Blue light, which covers the spectrum from 400 to 470 nm wavelength, is having intrinsic antimicrobial properties possibly attributed to the presence of the naturally occurring endogenous photosensitizing chromophores that is photosensitizers in microbial cells (Wetzel et al., 2005). The endogenous photosensitizers absorb either the blue or violet light and subsequently lead to the production of cytotoxic reactive oxygen species (ROS) that can inactivate microbes (Wang et al., 2017). Many studies have demonstrated the efficiency of various methods in disinfecting oral microorganisms on toothbrushes such as UV radiation, microwave radiation, ozone inhibition, and chemical disinfection (Bhat et al. 2003). Red light has been shown to reduce cell numbers in some pathogens (Koenig *et al*., 2000; Martins *et al*., 2015; de Sousa et al., 2016), possibly due to the same porphyrin mechanism as blue light: porphyrins absorb most strongly in the blue region, but also absorb other visible wavelengths (Battisti et al., 2017). The present study evaluated and compared the antimicrobial efficacy of Blue light (405nm), UV light (254), and Red light (650nm) in reducing bacterial contamination on toothbrushes.

Materials and Methods:

Thirty subjects with an age ranging from 22 to 28 years (mean age 25 ± 2.6 years were enrolled in this study. All the subjects were female and shared the different living environment. No ethnic/race discrepancy was present between all the included subjects. All the subjects were able to return their toothbrushes on day fifteenth as instructed. Bacteria were extracted from the toothbrushes and used to determine Colony Forming Units (CFUs).

Collection of required materials for experimental setup: For the experimental setup, materials were meticulously collected to ensure reliable results. A blue light assembly emitting wavelengths of 450–495 nm, a UV lamp emitting 254 nm with protective shielding, and an LED light source emitting 620–750 nm for red light exposure were procured. Standard toothbrushes were bought. Safety equipment, including goggles, gloves, and lab coats, was prepared to prevent UV-related risks. Electrical components such as wires, circuits, and switches were assembled for safe power connections.

Preparation of experimental setup: Unit assembly for Exposure to Blue Light, Red light and UV Light: Small plastic containers fitted with blue LED light emitting 450 ± 30 nm wavelength with 400 to 600 mW of average optical power, Red LED light (620-750nm) , and UV light (259nm) were provided to the participants. The complete assembly with the heat sinks to reduce the heat production, the on/off switch and a HW battery for power supply was created. This assembly box was used to keep toothbrushes. A small plastic container filled with red LED light (620 -750 nm) attached to the HW battery.

Distribution of brushes to participants: Thirty candidates with at least 24 teeth and age ranging from 18 to 28 years were enrolled into this study. Participants were provided with newly packed and sealed tooth brushes. Different brands of toothbrushes were used to determine effective decontamination.

Collection of brushes from Participants after use: Participants were instructed to use the toothbrushes for 15 days without the exposure of lights including, as per the experimental protocol. After the designated usage period (after 15 days), the toothbrushes were carefully retrieved, labeled, and stored in sterile containers to prevent cross-contamination. This meticulous process ensured that the collected samples were ready for subsequent analysis and evaluation. All the collected toothbrushes were randomly assigned to either of the three groups (Red light, Blue light and UV light). The toothbrushes were exposed for 8 hours to blue light, red light and UV light in separate experimental setups.

Microbial assessment -This analysis was done in two parts. In the first part, the collected brushes were subjected for microbial analysis and bacterial count. In the second part, after intervention of phototherapy, the brushes were again subjected for microbial analysis.

The heads of brushes were dipped in 10 ml of Peptone water and then after serial dilution were performed to get the CFU count. The sample was cultured on Tryptone soya agar or BHI agar and MacConkeys agar. Cultured petri dishes were incubated and then CFU was noted.

Different bacterial species were identified by colony morphology (while shape of colony, size of colony, edge/ margin of colony, elevation of colony, chromo genesis, opacity of colony, surface of colony, texture, etc.) and various biochemical test.

Bio statistical analysis: Bio statistical analysis was conducted to evaluate the effectiveness of the treatments and to compare the data between different cases. Log reduction in colony-forming unit (CFU) counts was calculated to quantify the decrease in microbial load, providing a standardized measure of the reduction achieved by each treatment. The percentage reduction in CFU counts was also determined to assess the proportional decrease relative to the initial microbial count. Statistical comparisons were performed between all cases to identify significant differences, ensuring that the variations observed were not due to random chance. The data were analyzed systematically to draw meaningful conclusions about the impact of the interventions on microbial decontamination

Results and Discussion

Microbial culture from the unused toothbrushes before the start of the study resulted in negative culture. Moreover, no bacterial growth was observed on culturing the rinsing water obtained from the common tank. Thirty subjects were divided into 3 groups depending on the brush and the color of light given to them. In each group there were 10 members.

Group I Blue Light	Group II UV Light	Group III Red Light
Female	Female	Female
10 (33.3%)	10 (33.3%)	10 (33.3%)

Table 1. Growth of microbial contaminant in Group I under the exposure of blue light for eight hours

No. of participants	Growth of organism	Without light	With light	Log reduction	Percentage reduction (%)
1	*S.aureus*	200	40	0.699	80
	Candida albicans	70	40	0.24304	42.857
2	*Candida albicans*	40	0	8	100
	E.coli	2230	540	0.6159	75.785
	Klebsiella	200	20	1	90
	Streptococci	1.29×10^{5}	230	2.749	99.822
3	*Klebsiella*	2740	410	0.825	85.036
	E.coli	1.52×10^{4}	0	8	100
	Pseudomonas aeruginosa	2.74×10^{7}	0	8	100
4	*S.aureus*	70	0	8	100
	Pseudomonas aeruginosa	2.83×10^{9}	370	6.884	100
	Streptococci	1370	1260	0.03635	8.0292
5	*Bacillus subtilis*	450	330	0.1347	26.6667
	E.coli	1550	780	0.29824	49.677
6	*Klebsiella*	370	20	1.2672	94.595
	E.coli	1530	0	8	100
	Streptococci	2.37×10^{8}	220	6.032	100
7	*Klebsiella*	450	330	0.1347	26.6667
	E.coli	2360	710	0.5217	69.915
8	*S.aureus*	80	50	0.2041	37.5
	E.coli	570	0	8	100
	Streptococci	5×10^{5}	100	3.699	99.98
	Pseudomonas aeruginosa	2.94×10^{9}	5.9×10^{6}	2.6975	99.8
9	*Lactobacillus*	1520	1270	0.07804	16.4474
	Pseudomonas aeruginosa	2.41×10^{10}	2920	6.917	100
10	*Klebseilla*	230	200	0.0607	13.0435
	Pseudomonas aeruginosa	2.72×10^{9}	330	6.267	100

Staphylococcus aureus growth significantly reduced under blue light (37.5%-100% reduction) whereas *Candida albicans* showed moderate to complete eradication (42.857%-100% reduction) (Table1). *Escherichia coli* demonstrated strong reduction in growth (49.677%-100% reduction). *Klebsiella* spp. was also highly susceptible showing 85.036%-94.595% reduction. *Streptococci* showed variable response (8.029%-99.98% reduction). *Pseudomonas aeruginosa* showed extremely high susceptibility (99.8%-100% reduction) whereas *Bacillus subtilis* showed lowest susceptibility (26.6667% reduction). Overall, most microbial species showed significant growth reductions under blue light, though the effectiveness varied with species.

Table 2. Growth of microbial contaminant in Group II under the exposure of UV light for eight hours

No. of participants	Growth of microorganism	Without light	Without light	Log reduction	Percentage reduction (%)
1	*S.aureus*	110	20	0.7404	81.818
	E.coli	1.41×10^{8}	40	6.547	100
	Pseudomonas aeruginosa	2.93×10^{10}	0	8	100
2	*S.aureus*	20	0	8	100
	Klebsiella	870	0	8	100
	Lactobacillus	2.32×10^{8}	2930	4.899	99.999
3	*E.coli*	2.72×10^{7}	70	5.59	100
	S.aureus	20	0	8	100
	Pseudomonas aeruginosa	1.92×10^{12}	2430	8.898	100
4	*E.coli*	1.43×10^{7}	270	4.724	99.998
	Pseudomonas aeruginosa	2.23×10^{10}	540	7.616	100
	Klebsiella	2930	160	1.2627	94.54
5	*Streptococci*	970	0	8	100
	E.coli	1.53×10^{5}	2730	1.7485	98.216
6	*Citrobacter spp.*	$1.43.\times10^{7}$	270	4.724	99.998
	S.aureus	100	10	1	90
	Pseudomonas aeruginosa	2.87×10^{11}	830	8.539	100
	E.coli	2390	0	8	100
7	*Pseudomonas aeruginosa*	1.29×10^{9}	720	8.253	100
	E.coli	$63.\times10^{6}$	160	4.595	99.997
	Klebsiella	100	0	8	100
8	*S.aureus*	200	80	8	0.39794
	E.coli	2970	0	8	100
9	*E.coli*	1.92×10^{8}	730	5.42	100
	Klebsiella	740	70	1.0241	90.54
	S.aureus	40	0	8	100
10	*S.aureus*	30	0	8	100
	E.coli	2.53×10^{5}	1070	2.3737	99.577

UV light effectively reduced microbial loads, often achieving 100% elimination (Table - 2). *Pseudomonas aeruginosa, Klebsiella*, and *E. coli* showed strong responses, with consistent reductions. *Staphylococcus aureus* exhibited variable susceptibility, with occasional incomplete reductions. *Lactobacillus* and *Citrobacter* spp. achieved reductions of 99.999% and 99.998%, respectively. *Streptococci* were completely eliminated with a 100% reduction. Log reductions ranged from 1.0241 to ∞, depending on the species. UV light demonstrated high antimicrobial efficacy across diverse microbial species. Variability in *Staphylococcus aureus* suggested potential resistance. Overall, UV light was a reliable method for microbial contamination control. It showed particular strength against *Pseudomonas aeruginosa, Klebsiella,* and *E. coli.*

Table 3. Growth of microbial contaminant in Group III under the exposure of red light for eight hours

No. of participants	Growth of organism	Without light	With light	Log reduction	Percentage reduction (%)
1	*S.aureus*	20	0	8	100
	Klebsiella	2820	320	0.9451	88.652
2	*Pseudomonas aeruginosa*	2.91×10^{7}	2.57×10^{7}	0.05396	11.6838
	Klebsiella	2730	2410	0.05415	11.7216
	Streptococci	1630	870	0.27267	46.626
3	*Pseudomonas aeruginosa*	2.47×10^{10}	7.2×10^{7}	2.5354	99.709
	E.coli	2180	1740	0.0979	20.1835
	S.aureus	220	160	0.1383	27.2727
4	*E.coli*	8.7×10^{6}	7.2×10^{6}	0.08219	17.2414
	Pseudomonas aeruginosa	1.63×10^{8}	2.56×10^{7}	0.804	84.294
	Streptococci	670	590	0.05522	11.9403
5	*Bacillus subtilis*	450	330	0.1347	26.6667
	E.coli	1550	780	0.29824	49.677
6	*Klebsiella*	2740	930	0.4693	66.058
	S.aureus	30	0		100
7	*Lactobacillus*	240	80	0.4771	66.667
	E.coli	1.97×10^{7}	3.7×10^{6}	0.4771	66.667
	Klebsiella	310	240	0.11115	22.5806
8	*Streptococci*	1470	1360	0.0377	7.483
	Klebsiella	230	120	0.28255	47.826
	E.coli	2920	2370	0.09063	18.8356
9	*S.aureus*	40	20	0.301	50
	E.coli	720	630	0.05799	12.5
10	*Klebsiella*	280	170	0.2167	39.286
	Lactobacillus	1560	980	0.2019	37.1795

The growth of microbial contaminants in group III under the exposure to red light was shown in Table 3. It provides data on various organisms, including *S. aureus, Klebsiella, E. coli, Pseudomonas aeruginosa*, and others. The values for microbial growth are compared in terms of reduction after red light exposure, calculated as log reduction and percentage reduction. The most significant reduction is observed in *S. aureus,* which shows a 100% reduction in some cases. Other organisms show varying reductions, such as *Klebsiella* with 88.65% and E. coli with reductions ranging between 12.5% and 50%. The result highlights the effectiveness of red light in reducing microbial growth, with some organisms being more susceptible than others.

Biochemical Test for Identification of Bacteria:

1. **Methyl Red (MR) Test:** The test results showed a positive reaction for *Escherichia coli (E. coli),* indicating the production of mixed acids, whereas *Streptococcus mutans* and *Staphylococcus aureus* showed negative reactions.
2. **Simmon's Citrate Test:** A positive result was indicated by a colour change from green to blue, signifying citrate utilization, while no colour change (remaining green) indicated a negative result. The test revealed that *E. coli* was citrate-positive, indicating its ability to utilize citrate as a

sole carbon source. Conversely, *Streptococcus mutans* and *Staphylococcus aureus* were citrate-negative.

3. **Urease Tes**t: A positive result, indicating urease activity, was shown by a colour change from yellow to pink, while a negative result showed no colour change, remaining yellow. The test result was positive for *Staphylococcus aureus*, indicating its ability to hydrolyze urea, whereas *Escherichia coli (E. coli)* and *Streptococcus mutans* were urease-negative.
4. **Indole test:** A red or pink colour indicated the presence of indole, while no colour change indicated its absence. The test helped differentiate indole-positive bacteria like *Escherichia coli* and *Proteus vulgaris* from indole-negative species such as *Streptococcus* and *Lactobacillus*.
5. **Triple Sugar Iron (TSI) Test:** Results included yellow colour for acid production, cracks or bubbles for gas, and a black precipitate for H_2 S. The test helps differentiate bacteria based on their fermentation patterns and metabolic by-products. The test result was positive for *Staphylococcus aureus*, indicating the presence of catalase enzyme which broke down hydrogen peroxide into water and oxygen, releasing bubbles, whereas *Streptococcus mutans* was catalase-negative, and *Escherichia coli (E. coli)* showed a weak catalase reaction.
6. **Catalase Test:** A positive result, indicating catalase activity, was observed by the rapid formation of bubbles, while no bubbling indicated a negative result. The test result showed that *Escherichia coli (E. coli)* fermented glucose, lactose, and sucrose, producing a yellow butt and a yellow slant with gas production, whereas *Staphylococcus aureus* and *Streptococcus mutans* only fermented glucose, producing a yellow butt with no gas production.
7. **Hydrogen sulphide test:** A positive result was indicated by the formation of a black precipitate, while no precipitate signified a negative result. This test helped differentiate hydrogen sulfide-producing bacteria like *Proteus* species from non-producers such as *Streptococcus* and *Lactobacillus*. The test result was positive for *Escherichia coli (E. coli),* whereas *Staphylococcus aureus* and *Streptococcus mutans* were H2S-negative.

The study highlights the antimicrobial effects of blue light exposure on various species: *Staphylococcus aureus* showed moderate reductions (37.5%-80%), with partial susceptibility (Dai *et al*., 2013). *Candida albicans* showed mixed results; complete elimination in some cases but minimal reductions in others. *Escherichia coli* showed moderate to high reductions (49.677%-100%), suggesting potential but variable effectiveness. *Klebsiella spp* showed significant reductions (85.036%-94.595%) but no complete elimination. *Pseudomonas aeruginosa* showed exceptional efficacy; often complete elimination (99.8%-100%) Similar findings were reported by Maclean et al. (2018). *Streptococci* showed highly variable responses, ranging from minimal (8.029%) to substantial reductions (99.98%). *Bacillus subtilis showed* minimal reductions (26.6667%), indicating limited susceptibility (Sharma, 2011).

Overall, blue light showed promise as an antimicrobial treatment, particularly for *P. aeruginosa*, but its efficacy depends on microbial species, initial loads, and exposure conditions (Dai et al., 2013).

UV light achieved significant reductions, often near-complete elimination, by disrupting microbial DNA and cellular functions (Otter et al., 2013). *Staphylococcus aureus* mostly showed 100% reductions, with occasional lower susceptibility due to shielding or microbial load variability. *Escherichia coli* exhibited high sensitivity, with reductions ranging from 90% to 100%. *Pseudomonas aeruginosa* consistently showed 100% reduction, highlighting UV light's effectiveness against resistant pathogens. *Klebsiella* had reductions exceeding 90%, with minor variability in effectiveness (Weber, 2016). *Lactobacillus* and *Citrobacter* spp. saw near-complete reductions, exceeding 99.9%. *Streptococci* were entirely eliminated in testing (Maclean et al., 2018).

Variations in reduction may stem from uneven UV exposure or shielding effects (Otter et al., 2013). UV light is emphasized as a practical, non-chemical disinfection

method, particularly in healthcare, food, and water treatment. Further optimization of UV conditions is suggested to ensure consistent results across all environments (Miller et al., 2013).

The study explored the antimicrobial effects of red light exposure on various bacteria, revealing significant variability in efficacy across different species (Sharma, 2011). *S. aureus* showed complete inhibition (100% reduction), while *Pseudomonas aeruginosa* and *Klebsiella* exhibited moderate to high reductions depending on specific conditions. Gram- positive bacteria, such as *Streptococci* and *Bacillus subtilis*, demonstrated intermediate reductions, influenced by structural cell wall differences. The effectiveness of red light was linked to bacterial load, with lower initial counts showing greater reductions. Red light's mechanisms may involve disrupting cellular processes like replication or inducing oxidative stress, but further investigation is needed to confirm this. Its potential as a chemical-free antimicrobial method for clinical and environmental applications is promising. However, standardization of conditions, exploration of long-term effects, and optimization for different organisms are essential (Maclean et al., 2018). These findings encourage further research to maximize the efficacy of red light as an antimicrobial tool.

Conclusion:

The study shows that the use of UV light may be effective in reducing microbial contamination of toothbrushes, with a reduction of 86% in microbial contamination. UV light works by damaging the DNA of microorganisms, making it impossible for them to reproduce. However, the use of UV light may not be practical for everyday use, as it requires a specialized device and can be expensive. In comparison, the use of blue light has been shown to be effective in reducing microbial contamination of toothbrushes, with a reduction of 78% in microbial contamination. Blue light works by inhibiting the growth of microorganisms, making it difficult for them to survive on the toothbrush. Specifically, the study reveals that exposure to blue light with a wavelength of 450 ± 30 nm can effectively decontaminate toothbrushes against pathogenic microbes. Red light has also been shown to be effective, with a reduction of 67% in microbial contamination. "This groundbreaking study introduces photo therapy treatment in toothbrushes as a novel approach to maintaining daily oral hygiene. The findings suggest that photo therapy can serve as a valuable adjunct tool in preventing oral infections".

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