Effect of Blue LED Exposure on the Antibacterial Activity of Curcumin

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Research Question

How does the exposure duration (0 h, 1 h, 2 h, 3 h, 4 h) (±0.1 s) of curcumin harvested from turmeric (*Curcuma longa*) to blue light-emitting diode (470 nm) from a distance of 0.5 cm affect its antibacterial activity measured through the number of colonies against *Escherichia coli* ATCC 25922 incubated at 28°C using the single plate-drop method?

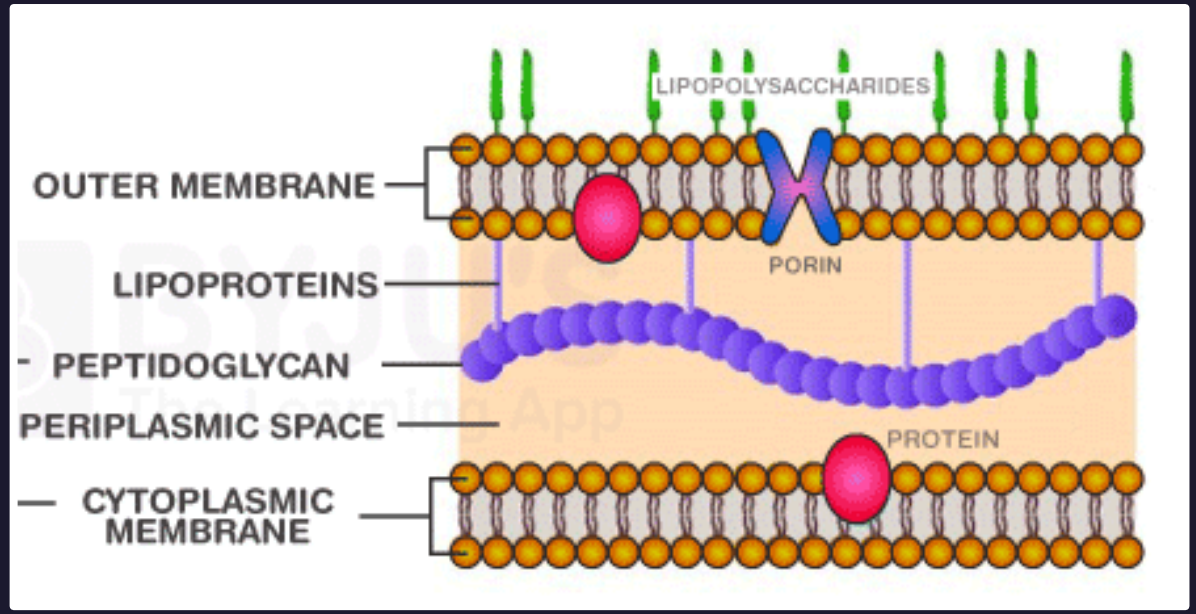
Objective of Investigation

- New solutions for antimicrobial resistance – one of the leading global health concerns!
- Using photodynamic therapy (PDT) via a safer alternative
- Exploiting antibacterial properties of curcumin despite its hydrophobicity that prevents its direct ingestion

Background Theory

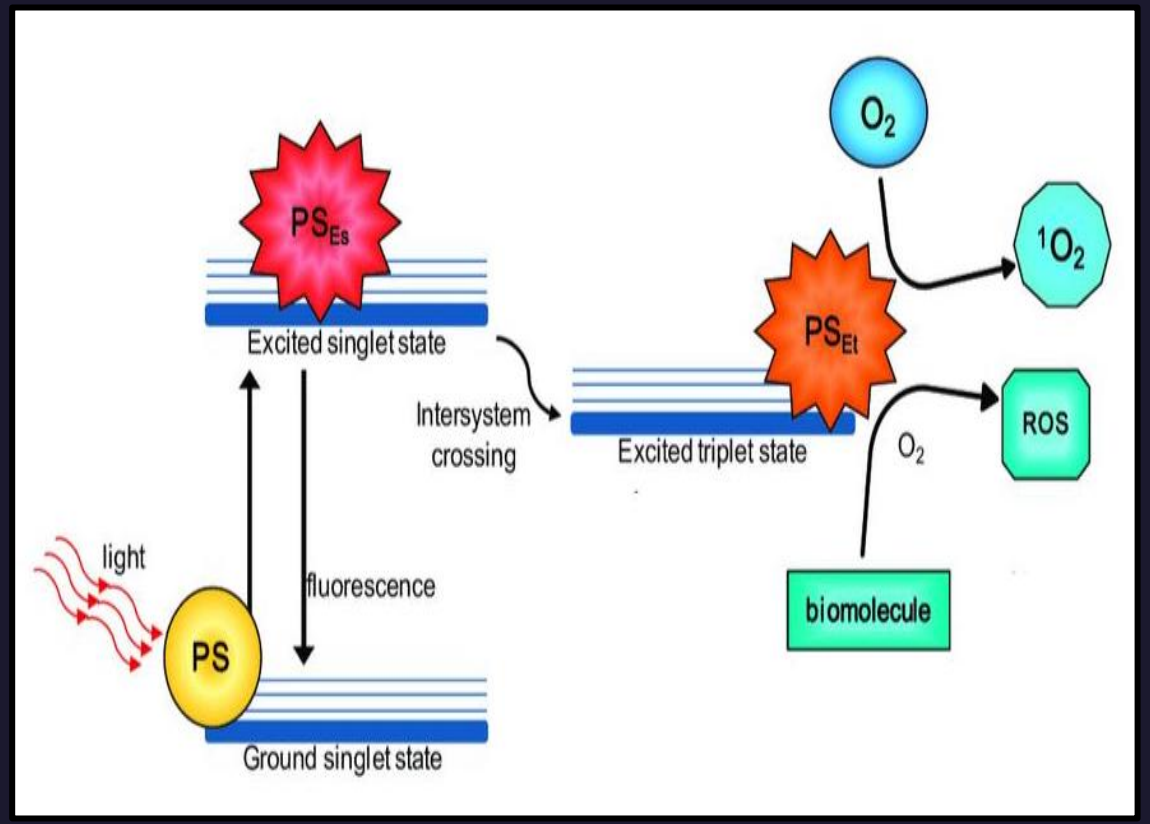
E. Coli ATCC 25922 bacterial strain

- Gram-negative strain – consists of thin peptidoglycan wall and surrounded by cell membrane
- Specialised channels known as porins that allow permeation of molecules
- Large molecular size of antibiotic molecules prevents its permation through porin
- Strain was chosen due to its non-pathogenicity and safety considerations



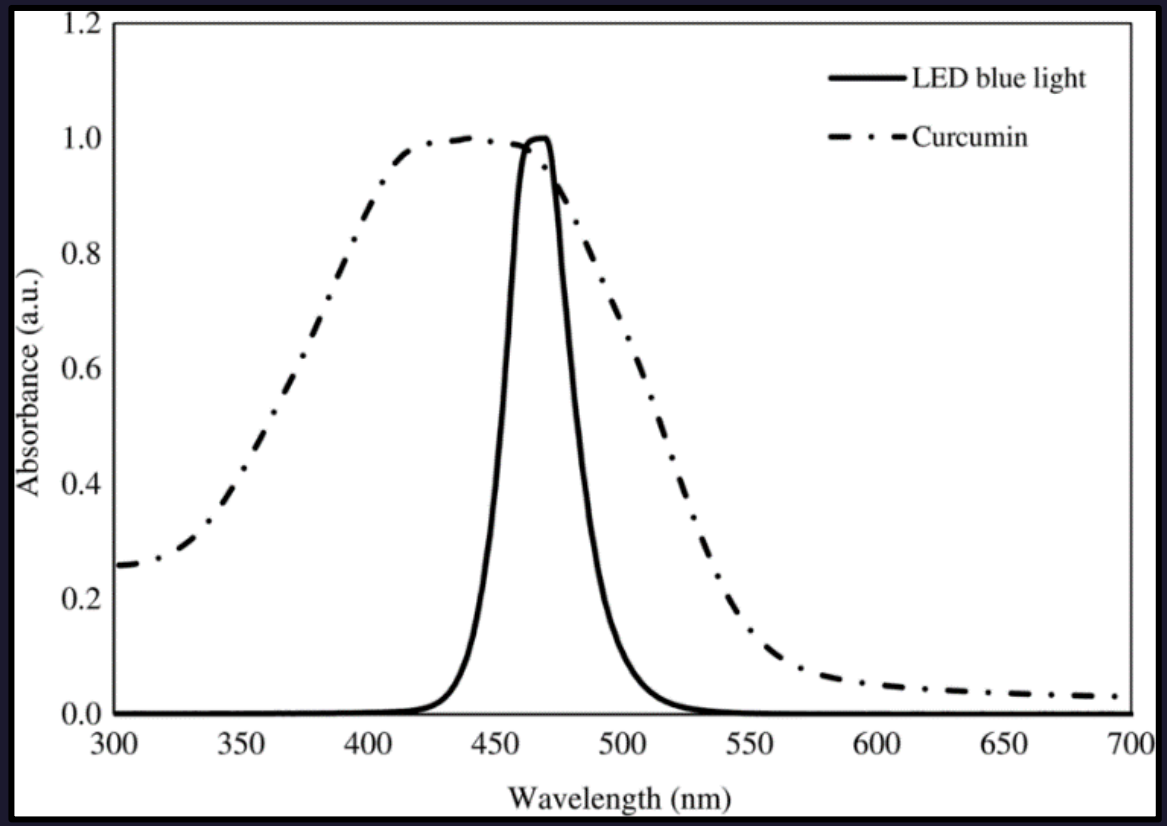
What is PDT? Why blue LED?

- High-energy wavelengths used to form reactive oxygen species and free radicals to kill pathogens and cancerous cells.
- Photosensitising compound that can be activated
  - Main application involves UV light – **RISKS DAMAGE TO LOCALISED HEALTHY TISSUE**
- Alternative using blue LED
  - Cucrumin has maximal absorbance wavelength at similar range as the wavelength emitted by blue LED (400-480 nm)
- Light exposure excites curcumin electrons to higher energy levels: singlet to triplet state
- PS3 electrons react with triplet oxygen to produce toxic singlet oxygen + hydroxyl radicals



Photosensitising properties of curcumin

- ROS accumulates in extracellular environment – flows down concentration gradient into cell
- ROS is needed for maintaining cell integrity
  - Excess concentration induces oxidative stress
- Oxidation of fatty acids (lysis) and DNA damage to regulatory sequences that code for basic cellular functions.
- Exclusive curcumin ROS with lifetime of 27 seconds! Longer reactivity likely to further damage cell
- Multitargeted mechanisms – less likely for resistance



Methodology

Brief overview

- Combining prepared curcumin and bacteria culture solutions
- Positive and negative controls
  - Ciprofloxacin and ethanol
- Exposure to different durations of blue LED
- Serial dilution + plated using single drops
- Counting number of colony-forming units (CFUs) and extrapolating value of original count from original sample volume

$$c = \frac{no.of\ colonies \times DF_T}{V}$$

Equation 1. Formula for calculating the concentration of colonies in the original sample.

where, $\boldsymbol{c}$ = concentration of colonies (colonies mL^{-1})

$\boldsymbol{no.ofcolonies}$ = number of colonies visible on the agar plate

$\boldsymbol{DF_T}$ = total dilution factor

$\boldsymbol{V}$ = volume of sample plated (mL)

$$DF_T = \frac{V_f}{V_i}$$

Equation 2. Formula for calculating the total dilution factor where the colonies were counted from.

where, $\boldsymbol{DF_T}$ = total dilution factor

$\boldsymbol{V_f}$ = final volume: volume of diluent and original sample transferred (mL)

$\boldsymbol{V_i}$ = initial volume: volume of original sample transferred (mL)

Variables

Independent variable

Exposure duration to blue LED (0 h, 1 h, 2 h, 3 h, 4 h) (± 0.1 s)

- 100 μM curcumin solution is exposed to blue LED (470 nm) for varying time periods. The time period is measured using a digital stopwatch, and the solutions are exposed 0.5 cm away from the light source in a dark environment.

Dependent variable

Number of Colonies in Original Sample

- The original sample is serially diluted by tenfold magnitude. Its colony count is determined by counting visible colonies from the plated dilutions and applying Equations 1. and 2. as explained in the Background Theory to calculate the total count in the original sample.

Hypothesis

Alternative Hypothesis (H1)

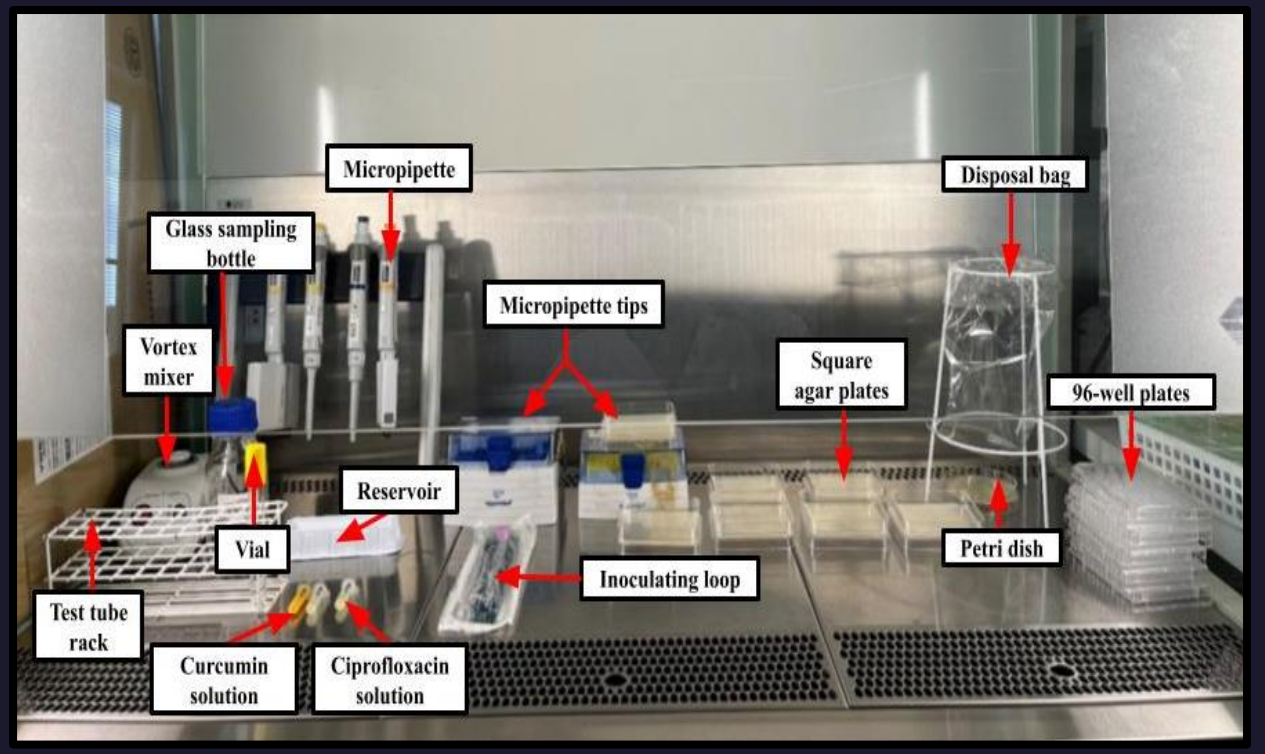
- Increasing blue LED exposure duration will decrease the colony count, thereby enhancing curcumin's antibacterial activity.
- Longer exposure provides increased energy that produces more PS3 electrons and higher ROS concentrations.
- Bacterial damage extent is larger, thereby forming fewer colonies in sample.
- Inevitable decrease in colonies for the non-exposed sample (i.e. 0h-sample) due to curcumin's existing antibacterial properties. Sample's colony number will demonstrate the current degree of activity naturally exerted by curcumin.

Null Hypothesis (H0)

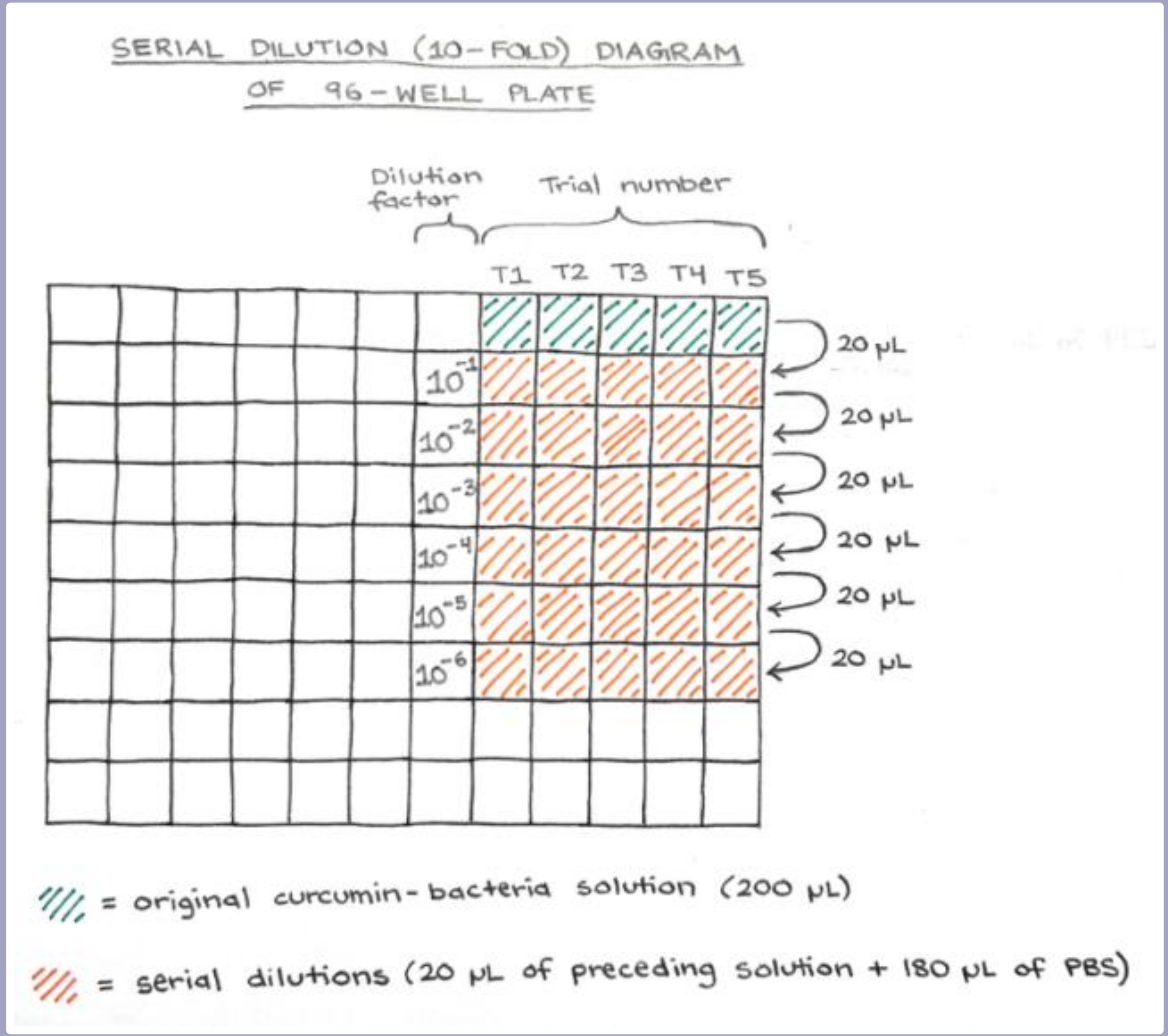
- No difference in colony count with increasing exposure duration.
- Count only affected by curcumin's existing antibacterial activity, so all samples will have identical counts to the non-exposed sample.

Methdology

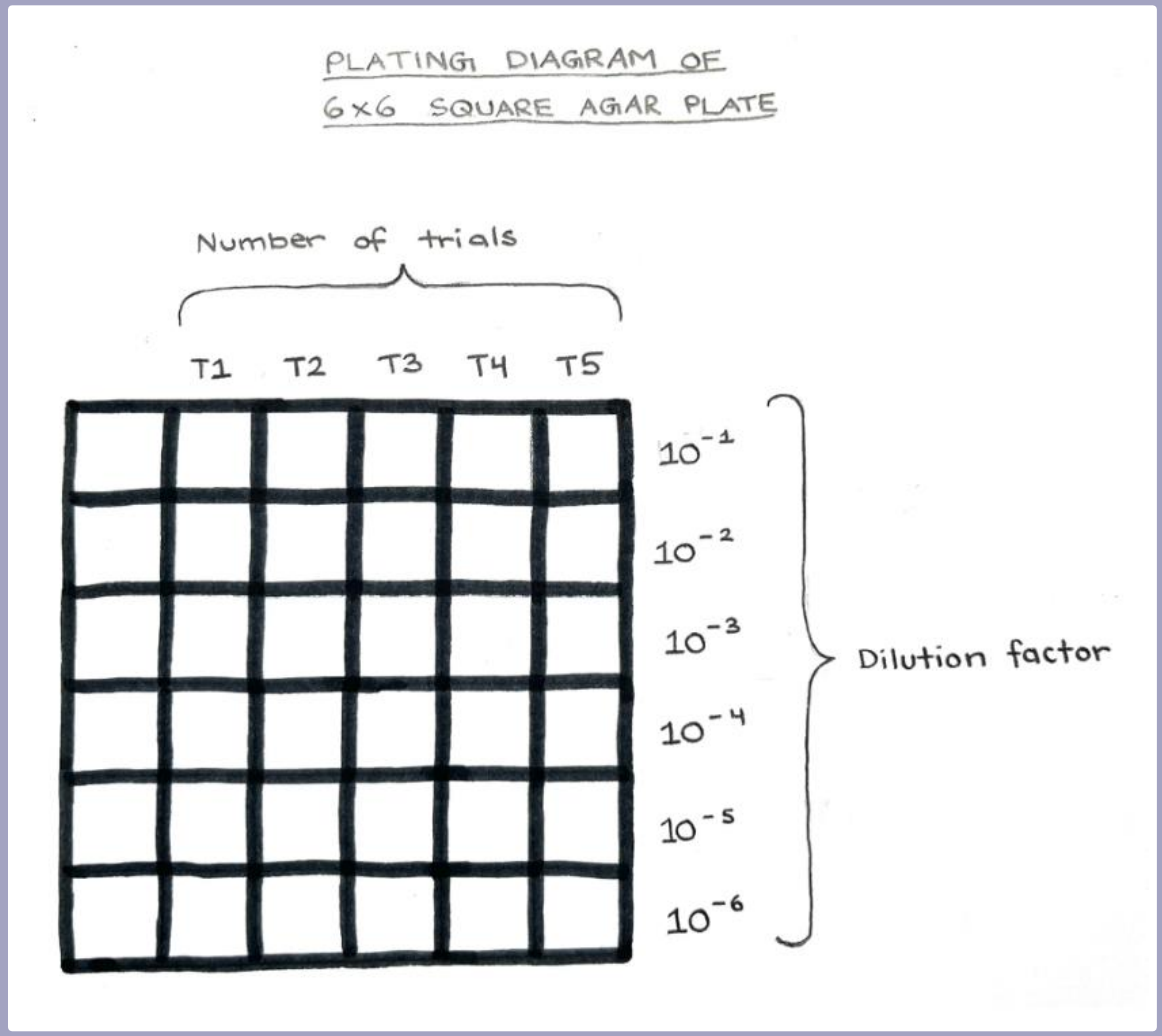
1. Preparing a 100µM curcumin solution using 99.5% ethanol as solvent
2. Preparing E.coli ATCC 25922 bacteria culture using PBS as solvent with colony concentration of 3.0×10^7 colonies mL^{-1}.
3. Preparation of agar plates and ciprofloxacin solution as positive control with 100µM concentration.
4. Exposure to blue LED in dark box.
5. Serially diluting curcumin-bacteria solution up to 10^{-6} concentration.
6. Single-drop plate method with 10 µM drops and five trials
7. Incubation for 24 hours at 37°C.



Plating diagrams



Serial dilution diagram



Agar plate diagram

Results and Analysis

Data analysis

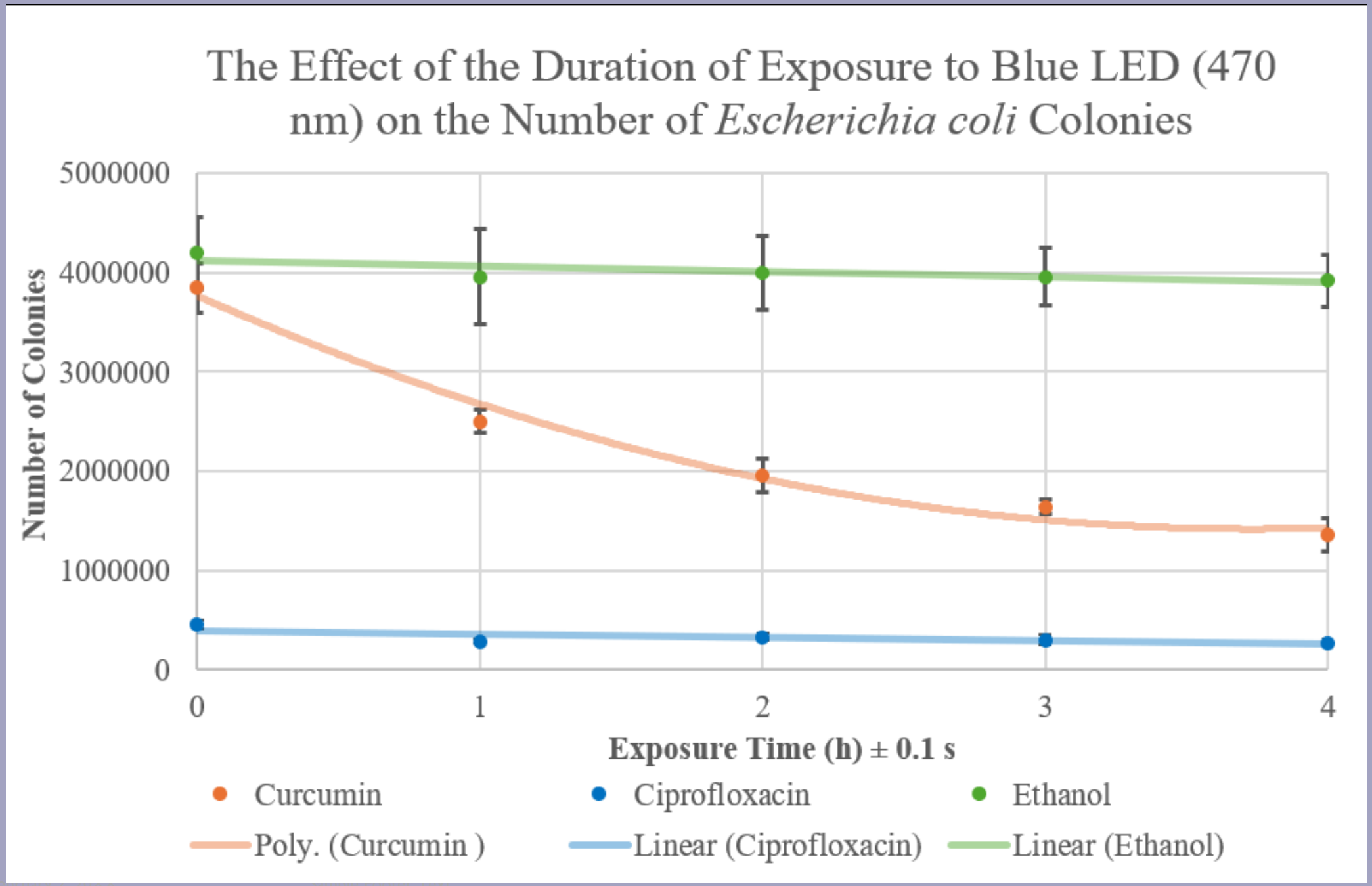
- Partially affirmed hypothesis – negative trend examined
  - 64.7% decrease in colony count
- Disproved that curcumin at 0h (no exposure) exhibited antibacterial activity
- Plateauing effect at three hours – photobleaching of curcumin
  - Dark period insulation between trials
- Increasing exposure showed decreasing colony diameters!
  - Circular colonies for 0 to 2 hours + punctiform colonies for 3 and 4 hours
- Alternative way of killing bacteria – decreasing colony count and area
- Ciprofloxacin control was only 8% lower than curcumin at 4 hours – can potentially be overcome

Qualitative data

- Colony size decreasing with increasing exposure duration
- Greater dispersion between colonies with increasing exposure
- Samples at 3 and 4 hours showed condensation – sign for production of heat that may have contributed to decreased cell count

Processed data

Exposure Time (h) ± 0.1 s	Average Colony Number	Standard Deviation	Standard Error	Total Colony Concentration (colonies mL^{-1})	Total Colony Number	Total Standard Deviation	Total Standard Error
0	19.3	2.75	1.23	1.93×10^{7}	3.85×10^{6}	5.51×10^{5}	2.46×10^{5}
1	12.5	1.29	0.58	1.25×10^{7}	2.50×10^{6}	2.58×10^{5}	1.15×10^{5}
2	9.8	1.92	0.86	9.80×10^{6}	1.96×10^{6}	3.85×10^{5}	1.72×10^{5}
3	8.2	0.84	0.37	8.20×10^{6}	1.64×10^{6}	1.67×10^{5}	7.48×10^{4}
4	6.8	1.92	0.86	6.80×10^{6}	1.36×10^{6}	3.85×10^{5}	1.72×10^{5}

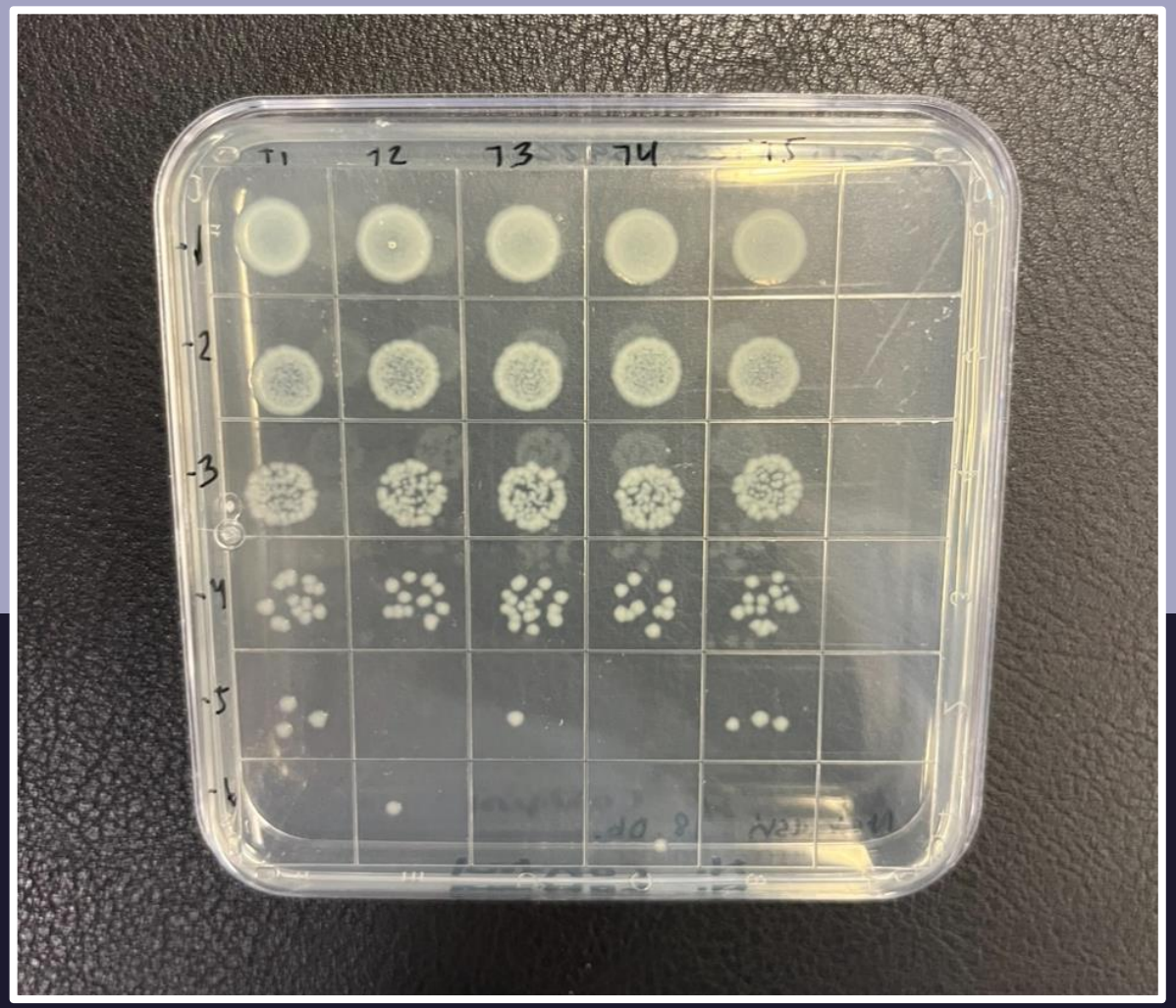

The Effect of the Duration of Exposure to Blue LED (470 nm) on the Number of *Escherichia coli* Colonies
Number of Colonies
5000000
4000000
3000000
2000000
1000000
0
0
1
2
3
4
Exposure Time (h) ± 0.1 s
Curcumin
Ciprofloxacin
Ethanol
Poly. (Curcumin)
Linear (Ciprofloxacin)
Linear (Ethanol)

Statistical test

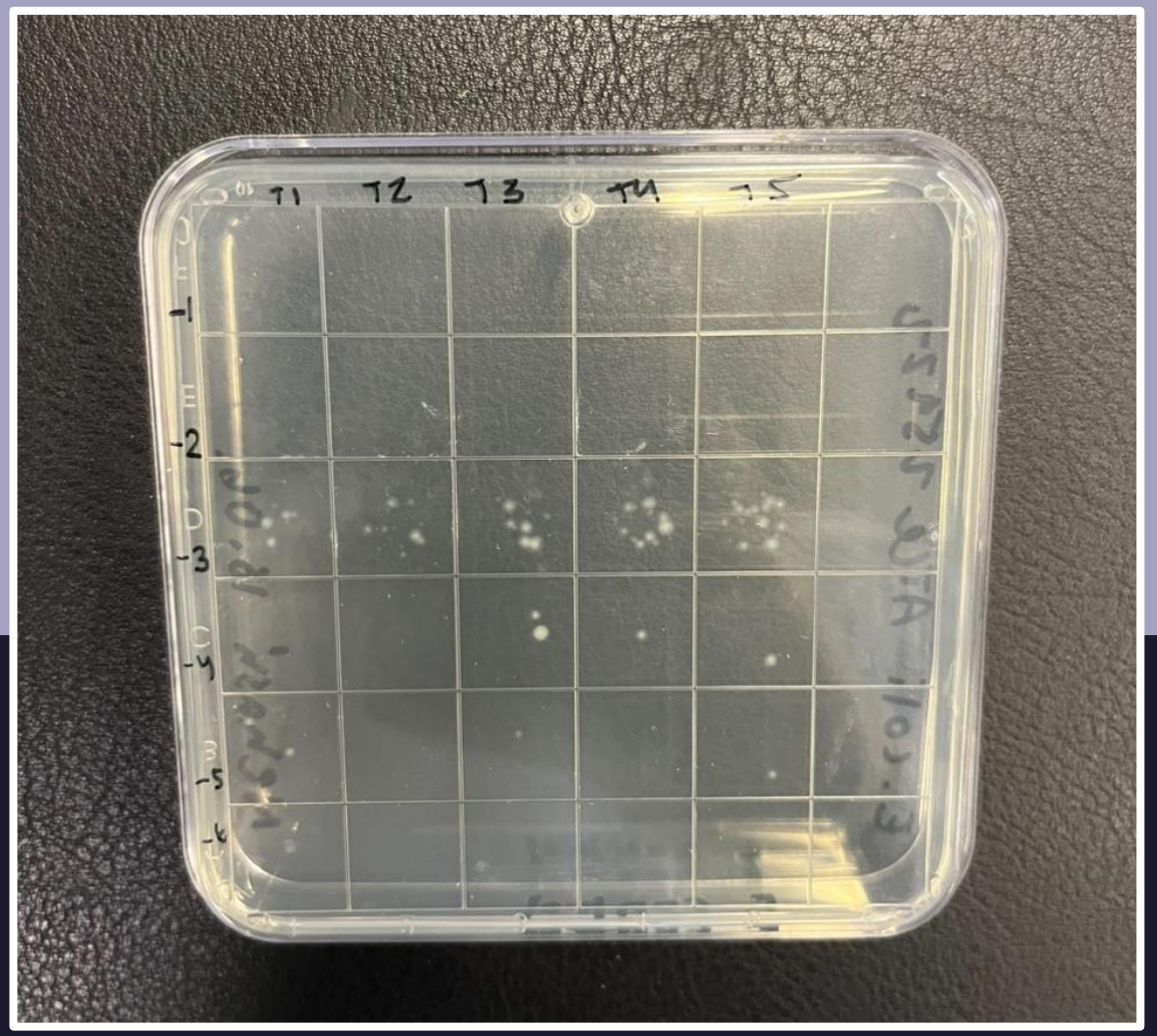
- One-tailed unpaired t-test using 95% confidence interval
- Compares means of curcumin and ethanol group for each exposure duration
- 0 hour duration strengthens conclusion that curcumin alone does not have antibacterial effect – must be induced!
- All other durations show statistical significance

Table 7. One-tailed unpaired t-test results of the change in the colony number between the curcumin treatment group and ethanol control over varying exposure durations.

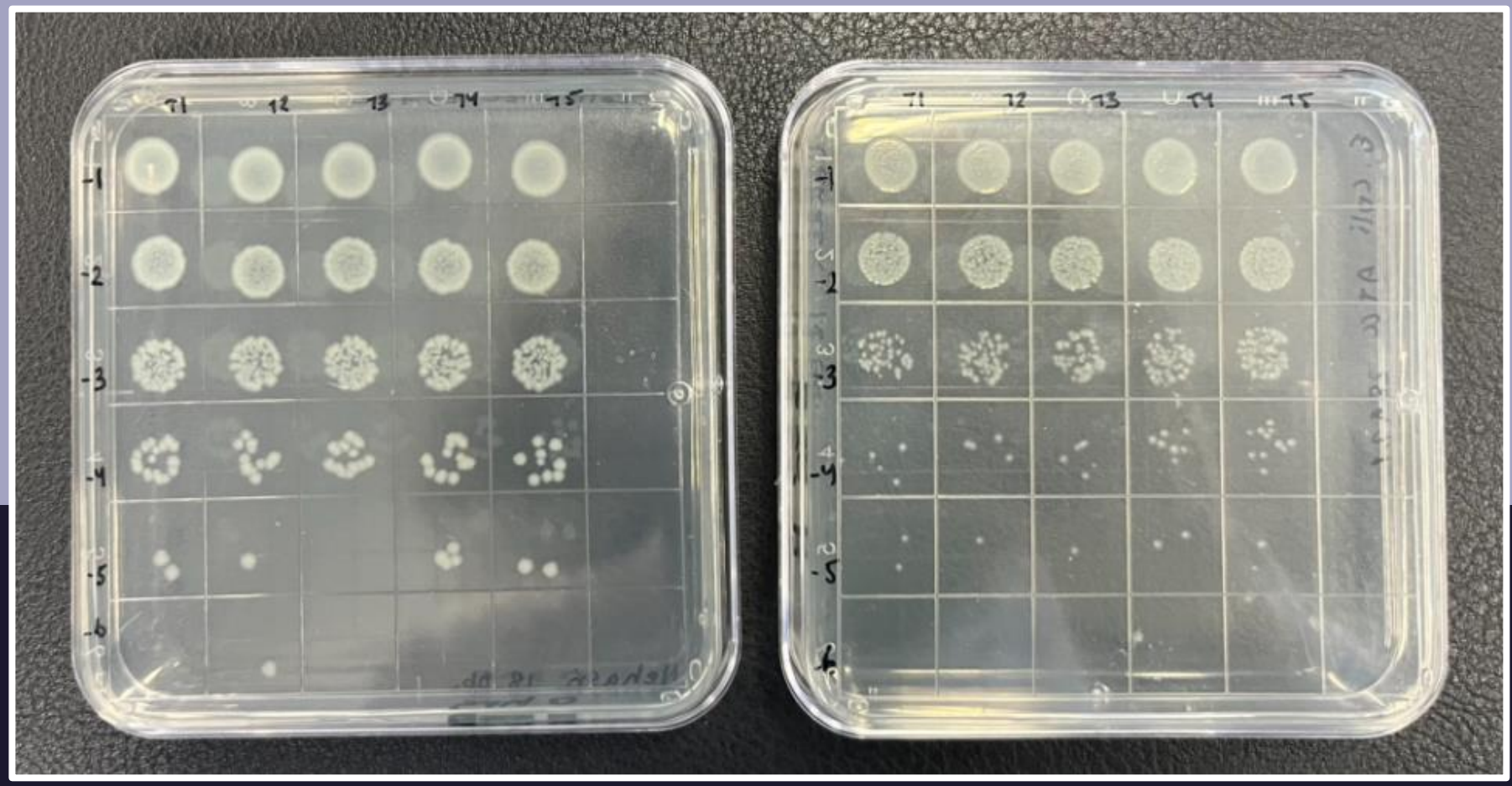
Curcumin vs. Negative Control (Ethanol)					
Result	**Exposure Time (h) ± 0.1 s**				
	0	**1**	**2**	**3**	**4**
p-value	0.133	0.013	0.003	0.002	< 0.001
Outcome	Insignificant	Significant	Significant	Significant	Very significant



Ethanol control at 4 hours



Ciprofloxacin control at 4 hours



Curcumin at 0 hours

Curcumin at 4 hours

Evaluation

Limitations

1. **Clustered colonies –Light source producing heat** – observed in qualitative observations
2. **Oxygen supply as limiting factor –Premature curcumin degradation** – solution was not stored in the dark, causing curcumin to lose electrons to visible light. Shortens maximum activity duration
3. **Not considering colony area** – decreasing diameter of colonies were observed, meaning that antibacterial activity measured underestimates actual value since area was neglected.
4. **Heat production** – observed in qualitative data that may have contributed to bacterial death.
5. **Premature degradation** – curcumin degrades under visible light, causing electrons to already be emitted (slow compared to blue LED). Solutions were not stored in dark container.
6. **Assuming colony represents single CFU** – CFUs can sometimes consist of multiple progenitor cells, may have skewed data that overestimates curcumin activity.

Thank you!