

## Photoeradication of Aquatic Pathogens by Curcumin

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### Abstract:

Photochemistry becomes the basis of the sustainable development goals 6 and 7 for clean water and energy. Removing aquatic pathogens is a global requirement to protect humans and aquatic animals from infectious diseases and emerging antibiotic resistance. Photodynamic antimicrobial chemotherapy combining photosensitizers and light sources is a greener approach for fighting such pathogens. A photosensitizer drug produces reactive oxygen species (ROS), rapidly disinfecting its local environment. Here, the ROS reacts with and disrupts the cell walls of the pathogens, apparent from singlet oxygen assays, superoxide assays, spectroscopic analyses, cell viability assays, and scanning electron microscopy imaging. The current study demonstrates disinfection of the aquatic pathogens *Vibrio (V.) cholerae*, *V. harveyi*, and *V. campbellii* by applying curcumin, a natural antioxidant in foods and light. Photoactivation of curcumin enhances ROS production and enables *V. cholerae* and *V. harveyi* disinfection within minutes at submicromolar concentrations. *V. campbellii* shows higher resistance to curcumin than *V. cholerae*, *V. harveyi*, which is due to the low affinity of the cell wall of the former for curcumin. The current study demonstrates curcumin's antimicrobial activity proposes its potential for water purification.

**Keywords:** Water purification, photodynamic therapy, antimicrobial, sustainable development goals, diarrhea

**Synopsis:** Waterborn pathogens are eradicated by antimicrobial phototherapy using faint blue light and a natural photosensitizer.

## Introduction

Photodynamic antimicrobial chemotherapy (PACT) received much attention as a replacement to antibiotics in several areas such as curing skin and periodontal infections,<sup>1-9</sup> removing food pathogen,<sup>10-11</sup> inactivating antibiotic-resistant pathogens,<sup>12</sup> deactivating bacteria of domestic animals,<sup>13-17</sup> and disinfecting textiles and polymers.<sup>18-19</sup> The ability of certain chromogenic molecules to generate reactive oxygen species (ROS) on photoexcitation, enabling them to be efficient photosensitizer (PS) drugs. Although PACT is a promising clinical treatment modality with minimal microbial resistance development, the responses of different bacteria towards a photoactivated PS vary depending on the ability of a PS to interact with pathogens and antioxidant mechanisms of bacteria.<sup>20-22</sup> It is essential to analyze the responses of different pathogenic and non-pathogenic microbes of clinical and environmental origins before PACT becomes significant to human and veterinary medicine and water/food disinfection.

Molecules of natural and synthetic origins and nanoparticles and their conjugates have been extensively investigated as PS drugs for photodynamic cancer therapy. Also, these PS drugs control oral pathogens, cure skin infections, self-sanitize clothes and face masks, disinfect water, and control aquatic pathogens.<sup>23</sup> Dyes like Rose Bengal, toluidine blue, and methylene blue for microbial and animal/human cell/tissue staining and imaging are PS drugs. They are effective against antibiotic-resistant pathogens like *Enterococcus faecalis*, *Streptococcus* spp., and *Vibrio* sp.<sup>1,13,24</sup> Also, synthetic derivatives of naturally occurring molecules like porphyrins and phthalocyanines are PS drugs in treating peripheral cancers and aquatic bacterial pathogens.<sup>25-27</sup> The search for novel PS drugs continues for more efficient ones with high ROS production, tunable optical absorption, insignificant dark toxicities, and treating Gram-negative bacteria. Photoactivated nanomaterials such as carbon nanotubes, fullerenes, graphene, gold, TiO<sub>2</sub>, and ZnO kill various pathogens in the fungal and bacterial families by destroying or denaturing DNAs or cell membrane.<sup>23</sup> Several plant extracts have been recently reported to produce ROS and kill bacterial pathogens on photoexcitation.<sup>28</sup> Among natural products, curcumin and riboflavin are promising PS drugs for photodynamic eradication of foodborne pathogens like *Salmonella* sp and *V. parahaemolyticus*.<sup>29-31</sup> Curcumins are yellow-colored and sparingly water-soluble polyphenolic pigments extracted from the root tuber *Curcuma longa* (*Zingiberaceae* family) and are optically active under blue light (420 - 460 nm) excitation. Although curcumins infiltrate into cancer phototherapy, their potentials for water disinfection and PACT are yet to be systematically analyzed.

We report the PACT efficacy of curcumin as a PS drug for killing water-associated pathogens such as *V. cholerae*, *V. campbellii*, and *V. harveyi* isolated from clinical and aquaculture samples. *Vibrio*

spp. causes diarrheal disease in humans<sup>32</sup> and vibriosis in aquatic organisms.<sup>13, 33</sup> There are nearly twelve pathogenic vibrio species, among which *V. cholerae* is the clinical pathogen that causes around 2.9 million diarrheal cases and 95000 deaths annually worldwide.<sup>34</sup> The burden of water-associated diseases is more in developing economies where quality drinking water is scarce. These pathogens are transmitted through contaminated food and drinking water, and many of them are resistant to multiple antibiotics. World Health Organization has proposed eliminating the transmission of cholera (caused by *V. cholerae*) by 2030. The supply of safe drinking water is the sustainable development goal 6 (SDG). We investigate the antibacterial effect of curcumin before and after photoexcitation with blue LED ( $\lambda_{\text{ex}}$ : 460 nm) from the viewpoint of its efficiency to produce ROS and bind different *V. spp.*

## Results And Discussion

Curcumins are known for their antimicrobial activity against several fungal, bacterial and viral pathogens. Their use as a preservative of food and gastrointestinal disease has been proposed in the past.<sup>35-40</sup> In the present study, we used curcumin as a PACT drug against pathogenic *Vibrio* spp. and found that the PACT activity increased significantly on photoactivation. Fig. 1A, B shows the absorption and emission features of the natural curcumin in phosphate-buffered saline (PBS). It has a broad absorption spectrum extending in the UV-Vis region. However, we find the absorption and emission features differently in a nonpolar solvent like toluene (Fig. 1C, D). The fine vibronic absorption and emission features in toluene disappear in the aqueous buffer, showing the changes to the HOMO-LUMO levels. Similar changes in the conformation of curcumin were observed in response to solvent characteristics, temperature, and substitutions in curcumin. Also, the keto-enol tautomerism of the  $\beta$ -diketone structure in a solution contributes to such spectral characteristics.<sup>41</sup> Detailed investigations of the photophysical properties of photoexcited curcumin confirmed the predominance of the enol form in a solution, which is prone to isomeric changes and it shows different optical properties.<sup>41</sup> We examined the role of photoirradiation of curcumin on its emission and absorption features. While its absorption spectra (Fig. S2) remained intact under dark, the absorbance and emission intensities were decreased under continuous excitation with blue light (10 mW cm<sup>-2</sup>, 60 min) from an LED (Figs. 1B, D, S1, and S2), which is due to the self-sensitized pseudo unimolecular degradation by electron transfer from the excited state to molecular oxygen.<sup>42,43</sup> While such photosensitized oxygenation of curcumin is a dominant degradation pathway, several photoactive intermediates and products in the electron transfer- or ROS-mediated degradation make it challenging to assign the antioxidant properties to the parent curcumin or one of the intermediates/photoproducts.<sup>43</sup> Also, the role of ROS in the photodegradation of curcumin is considered from previous reports

showing an increased degradation rate in the presence of ROS producers, silver nanoparticles,<sup>44</sup> and TiO<sub>2</sub>,<sup>45</sup> and reduced degradation with ROS quenchers such as NaN<sub>3</sub>, KI, and methyl viologen (MV<sup>2+</sup>).<sup>45</sup> Furthermore, vanillic acid, ferulic acid, vanillin, and cinnamic acid were identified as the photoproducts of curcumin, which are nontoxic.<sup>45-48</sup>

We examined the PACT effect of curcumin by photoirradiation of bacterial suspensions supplemented by curcumin (Fig. 2). Bacteria in a suspension before and after the treatment were grown over the Luria Bertani (LB) agar plate surface. The PACT efficiency was determined from the number of survived bacteria. The photoexcited curcumin had higher antimicrobial activity against pathogenic *Vibrio* spp. (Fig. 2) than a bacterial suspension in the dark. The survival rate of all *Vibrio* spp. has decreased on exposure to curcumin and the light from a blue LED (11 mW cm<sup>-2</sup>). Here the irradiation intensity was kept constant for identifying the minimum curcumin concentration for appreciable cell death. The cells of *V. harveyi* and *V. campbellii* survived up to 5 μM of curcumin solutions under dark conditions. At the same time, the survival rate of *V. cholerae* began falling with 0.5 μM of curcumin solution. In the dark, nearly 80 % of *V. cholerae* could be disinfected by a curcumin solution (5 μM). Still, such a reduction was not observed for *V. harveyi* or *V. campbellii* under similar conditions. The survival rate of *V. cholerae* and *V. harveyi* has reduced to below 10 % at 0.5 and 0.1 μM concentrations of curcumin, respectively, under photoirradiation. However, *V. campbellii* was more resistant, and nearly 35 % of cells survived with a curcumin solution (5 μM) under photoexcitation. These results suggest submicromolar curcumin and photoirradiation effectively eradicate *V. cholerae* and *V. harveyi*.

Antimicrobial activities of curcumin against various bacteria have been shown in the literature,<sup>39</sup> and the activity varies with the bacterial phenotype and curcumin concentration.<sup>49</sup> Relatively higher concentrations of curcumin were reported to induce membrane depolarization (12 μg mL<sup>-1</sup>), ROS accumulation (12 μg mL<sup>-1</sup>), DNA fragmentation, and membrane destabilization (24 μg mL<sup>-1</sup>) in *E. coli*.<sup>36</sup> Studying the antibacterial activity against 23 clinical isolates of Grampositive and Gramnegative bacteria, Betts *et al.*<sup>49</sup> showed a drastic difference in the inhibitory concentrations of curcumin against *E. faecium* and *P. aeruginosa* (174 and 1390 μM respectively). Interestingly the minimum inhibitory concentration (MIC) for this PS against *P. aeruginosa* varied from 1390 to 2780 μM. We also found that a 5 μM of curcumin solution inhibited the survival of more than 80 % of *V. cholerae* under dark conditions. Conversely, *V. harveyi* and *V. campbellii* survived at this concentration. This difference could also be due to the differences in the curcumin uptake abilities among different *Vibrio* spp.

We further investigated the abilities of curcumin to attach to the bacterial surface, produce ROS and disintegrate the bacterial cell walls. The uptake of curcumin varied among different species of vibrios (Fig. 3). We incubated bacteria with 10, 50, and 100 μM of curcumin in separate tubes for 30

min, and the attachment of curcumin was determined spectrophotometrically. Nearly 80 % of curcumin was retained on the surface of *V. cholerae* after 30 min of incubation, while it was much lower (<40 %) for *V. campbellii*. The cells of *V. harveyi* also retained nearly 70 % of the curcumin. Such a difference was more prominent between Grampositive and Gramnegative ones, where the compositions of peptidoglycans and lipopolysaccharides were significantly different. The differential antibacterial interaction of curcumin for Grampositive and Gramnegative bacteria was reported recently.<sup>39,50</sup> However, the difference between different species of the same organism is unknown.

We correlate the bacterial survival rate to the ROS production efficiency of curcumin. While electron transfer to oxygen and superoxide generation supplement the ROS pathways, the excited state properties such as the triplet energy (ca. 191 kJ mol<sup>-1</sup>), triplet quantum yield (ca. 0.11), and the triplet lifetime (ca. 1.5 μs) of curcumin support singlet oxygen generation.<sup>51</sup> The efficiencies of curcumin to generate superoxide and singlet oxygen under dark and photoexcitation were evaluated spectroscopically using the sensors *N*-[10-(9,10-dimethylanthracene-2-yl)-7-(dimethylamino)-5,5-dimethyldibenzo[*b,e*]silylium-3(5*H*)-ylidene]-*N*-methylmethanaminium-chloride (SiDMA), 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (INT) and 9, 10-anthracenediyl-bis(methylene)dimalonic acid (ADMA) (Figs. 4, S3). We used a mixture of riboflavin and methionine as the positive control. The formation of red-colored formazan by the reduction of INT was detected ca 505 nm, and the value is presented as the formation of superoxide radicals (Fig. 4A). We observed approximately a 700-fold increase in superoxide radical production by positive controls under photoexcitation. Conversely, curcumin did not produce appreciable amounts of superoxide. Singlet oxygen formation is evaluated by spectroscopically measuring the changes in the absorption (a continuous decrease) of ADMA (Fig. 4A) or SiDMA (Fig. 4B) at 400 nm or an enhancement in the SiDMA's fluorescence intensity ca 675 nm (Fig. 4C). This increase is due to the oxidation of anthracene in SiDMA and the uncaging of intramolecular electron transfer (Fig. 4D). Using ADMA, it showed ca. 36 % increases in singlet oxygen production under 460 nm photoexcitation. Positive controls also showed a 37.8 % increase in the production of singlet oxygen under 460 nm photoirradiation. Several strategies, including converting bulk molecules into nanoparticles,<sup>52</sup> conjugation with other antimicrobials like silver nanoparticles,<sup>53</sup> and photoexcitation<sup>54</sup> improve curcumin's antimicrobial activity. In the present study, blue-light photoexcitation enhances curcumin's singlet oxygen production and thus increases its PACT efficiency against *Vibrio* spp. The singlet oxygen production of curcumin was increased approximately six times on photoexcitation. Since more curcumin molecules are attached to the surface of *V. cholerae* and *V. harveyi*, the singlet oxygen toxicity and subsequent antibacterial activity would also be higher. Nearly all *V. cholerae* and *V.*

*harveyi* cells were killed on photoexcitation with 0.5 and 0.1  $\mu\text{M}$  curcumin solutions, respectively. Conversely, the *V. campbellii* poorly uptakes curcumin, decreasing the singlet oxygen toxicity and increasing the survival rate (40 %) even with a 5  $\mu\text{M}$  solution. The requirement to uptake an appreciable amount of a PS drug for killing Grampositive and Gramnegative ones was demonstrated earlier.<sup>55</sup>

The production of ROS<sup>56</sup> was confirmed by measuring the death rate of *V. harveyi* with the antioxidants melanin, and ascorbic acid (Fig. 5). The cells of *V. harveyi* could not survive in the presence of photoexcited curcumin, and a 100 % death was recorded. Interestingly, nearly 23 and 30 % of cells could survive curcumin and light in the presence of ascorbic acid or melanin (Fig. 5). Nevertheless, melanin or ascorbic acid does not quantitatively quench all ROS. The reactive oxygen molecules could destroy the inner cytoplasmic membrane and unsaturated fatty acids and protein of the outer membrane, thus impinging the cell membrane integrity.<sup>57</sup>

We evaluated the influences of photoexcited-curcumin to the cell membrane stability of *Vibrio* spp. from the cell survival rate and scanning electron microscopy images (Fig. 6). The cell wall integrity of *V. harveyi* and *V. campbellii* was maintained intact on incubation with curcumin under dark. The cell wall disruption was evident among those excited using blue light after adding curcumin. Also, the cell wall disintegration was different for *V. cholerae* exposed to curcumin and light. The lipopolysaccharide layer of Gram-negative bacteria is less amenable to singlet oxygen,<sup>57</sup> and variations in the thickness of this layer might have contributed to the survival of *V. campbellii*, *V. cholerae*, and *V. harveyi*. The curcumin nanoparticles attach to the surface of *Staphylococcus aureus*, which subsequently disrupts the peptidoglycan layer and kills the cells.<sup>52</sup>

## Summary

The singlet oxygen production ability of curcumin has been demonstrated earlier and prompted its potential application to cancer phototherapy,<sup>58</sup> treat oral infections,<sup>59</sup> degrade bacterial biofilms,<sup>60</sup> and decontaminate foods.<sup>17,61</sup> Studies reported the efficacy of PACT using Rose Bengal,<sup>13</sup> porphyrin derivatives,<sup>62,63</sup> and curcumin<sup>17</sup> as photosensitizers in inactivating aquatic pathogen *Vibrio* spp. UNICEF estimated that diarrhea alone causes 8% of all fatalities among children below 5 years.<sup>64</sup> Or the number hits 480,000 annually. Therefore, it is important to find alternative strategies to disinfect drinking water. PACT is a promising strategy to extend from water purification plants to PET bottles. PACT to drinking water purification was recorded in the 1970s, with PACT's disinfection of polio virus and other waterborne pathogens.<sup>65</sup> Recently, Ugwuja *et al.*<sup>28</sup> demonstrated the use of hybrid clay nano-composites containing the seeds of *Carica papaya* for water photo-disinfection. Concoctions of

curcumin were used recently in Asian countries to gain immunity against infections of SARS-CoV-2, even at higher concentrations.<sup>66</sup> Considering these facts, the finding of the impact of curcumin to PACT is promising for disinfection of water-associated pathogens.

The current work indicate that photoactivation enhances the antimicrobial properties of curcumin by the increased production of singlet oxygen. Here, curcumin attached to the surface of *Vibrio* spp. destabilizes the bacterial cell walls. Since curcumin is a commercial supplement for various health benefits, curcumin-based PACT can be a promising strategy for disinfecting water. This may support providing safe drinking water for all, thus avoiding water-associated diseases, and reducing antibiotics and chemicals in water treatment, reinforcing the SGD-6 pillar under the clean water program.

## **Experimental Section**

### **Microorganisms and photoexcitation experiments**

The isolates of *V. campbellii* (MMRF 1060), *V. cholerae* (MMRF 1099) and *V. harveyi* (MMRF 1107) used in the current study were obtained from Marine Microbial Reference Facility (MMRF) maintained at CSIR-National Institute of Oceanography. These bacteria were previously isolated from the stool of cholera-infected patients<sup>32</sup> and moribund shrimp samples.<sup>13, 33</sup> The isolates were revived from the lyophilized stocks following standard protocols, and working isolates were maintained in LB agar medium. The isolates' purity was verified by staining and analysis of profile of fatty acids. *Vibrio* spp. for further studies were grown in LB broth for 24 h in an incubator shaker maintained at  $28 \pm 2$  °C and 100 rpm.

### **Absorption and fluorescence spectral analyses**

We recorded the absorption and fluorescence spectra of curcumin (TCI, Japan or AVT natural products, India), SiDMA (Dojindo, Japan) in an Evolution 200 spectrophotometer and a Hitachi 4100 FL spectrometer. The absorption spectra of curcumin (10 or 15  $\mu$ M) in toluene or PBS before and after photoexcitation (10 mW  $\text{cm}^{-2}$ ) for 60 min were measured at equal time intervals. After recording the initial absorption spectrum, curcumin solution was exposed to light (10 mW  $\text{cm}^{-2}$ ) from a LED source ( $\lambda_{\text{max}}$  405 nm). The emission spectra of SiDMA in the presence of were collected before and after equal time intervals of photoactivation of coumarin.

### **Curcumin uptake by *Vibrio* spp.**

*V. campbellii*, *V. cholerae*, and *V. harveyi*, were incubated for 30 min with curcumin solutions having 10, 50, and 100  $\mu$ M concentrations and the absorption was monitored spectrophotometrically. Overnight grown *Vibrio* spp. (*V. campbellii*, *V. cholerae*, and *V. harveyi*) were dispersed in PBS (to

get a final OD of 0.1 at 600 nm) and supplemented with 10~100  $\mu\text{M}$  curcumin solutions at  $28 \pm 2$  °C, 30 min). Subsequently, we separated the pathogen cells by centrifugation ( $8 \times 10^3$  rpm; 10 min). Next, we measured the absorbance of the supernatant ca 420 nm and compared with the initial absorbance. The decrease in the absorbance indicates the successful bacterial uptake.

### ***In vitro* analysis on the antibacterial activities of photoexcited curcumin**

The role of curcumin photoexcitation on its antibacterial activity was studied using a death rate assay. Overnight grown *Vibrio* spp., were separated from LB broth, resuspended in phosphate-buffered saline, and diluted to get  $10^6$  cells  $\text{mL}^{-1}$ . This solution was distributed into sterile tubes and mixed with different concentrations of curcumin (0.001- 5  $\mu\text{M}$ ) to a final reaction volume of 2 ml. Separate tubes were maintained for each bacterium and concentrations of curcumin. Separate sets of tubes were maintained in the dark and under photoexcitation to differentiate curcumin's antimicrobial action (dark toxicity) with or without photoexcitation. A set of control without curcumin also was maintained. All experiments were conducted in triplicate. The tubes assigned for photoexcitation experiments were exposed to blue light for 30 minutes. An LED source was placed just above the surface of the bacterial solution, and the output power was measured as  $11 \text{ mW cm}^{-2}$ . The temperature of the reaction mixture was recorded continuously. 100  $\mu\text{l}$  samples were withdrawn from each tube before and after photoexcitation and spread on a standard LB agar plate. The plates were maintained in an incubator (28 °C) for 24 h. The newly appeared colonies were counted. The efficiency of the treatment was measured as the number of bacteria that survived after 30 minutes of treatment in comparison with the initial counts.

### **Photoexcitation and reactive oxygen species production by curcumin**

The production of superoxide radicals and singlet oxygen by curcumin and photoexcited curcumin were monitored spectrophotometrically using INT<sup>67</sup> and ADMA<sup>17</sup> probes, respectively. Riboflavin (240  $\mu\text{M}$ ) mixed with methionine (10 mM) was used as the positive control for superoxide and singlet oxygen generation in the assays.<sup>68</sup> Four sets of tubes containing curcumin solution (50  $\mu\text{M}$ ) were prepared, and two sets each were assigned for INT and ADMA assay, respectively. The tubes assigned for INT and ADMA assays were supplemented separately with 400  $\mu\text{l}$  of INT (0.2 mM) and 200  $\mu\text{l}$  of ADMA (100  $\mu\text{M}$ , 2 mL). One set of the tube from each group was maintained under dark conditions, and another set was exposed to blue light for 10 min. The production of superoxide radicals and singlet oxygen was measured separately as the formation of red-colored formazan (at 505 nm) and decrease in ADMA's absorbance (400 nm). Production of superoxide and singlet oxygen radicals was calculated in comparison with negative controls. Also, singlet oxygen production was assayed using the



fluorescence sensor SiDMA.<sup>69</sup> The fluorescence of such sensors<sup>70-72</sup> is quenched by an intramolecular electron transfer. Singlet oxygen produced by curcumin oxidizes anthracene in the sensor, blocks the electron transfer, and releases the fluorescence. We used SiDMA prepared in DMSO (1 $\mu$ M) and measured its fluorescence intensities at different time intervals under photoactivation of curcumin (10  $\mu$ M).

Overnight grown *V. harveyi* cells were prepared for death rate assay as prepared in the above session. The experimental tubes were supplemented with antioxidants (bacterial melanin (10 ppm) and ascorbic acid (100 ppm) and exposed to photoexcitation. *V. harveyi*'s deactivation rate after the photoexcitation with or without ascorbic acid and bacterial melanin was monitored. The abilities of photoexcited curcumin to produce reactive oxygen species were further confirmed by performing a death rate assay in the presence of antioxidants melanin and ascorbic acid.

### **The role of PACT on *Vibrio* spp.'s membrane integrity**

PACT effect on bacterial morphology was studied by scanning electron microscopy. Before and after photoexcitation for 30 min, the bacterial cells were prepared for the SEM analysis following standard protocols. Briefly, we used PBS and washed the cells, treated them with buffered glutaraldehyde (3 %) for one hour, and dehydrated them by exposure to increasing concentrations (1 min each in 30, 40, 50, 60, 70, 80, 90, and 100 %) of ethanol. The cells were further placed over a coverslip, air dried, sputter-coated, which was followed by collecting the SEM images on a JEOL-JSM- 6490 microscope.

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### **Author Information**

### **Author Contributions**

A. A. and V. B. led the project. A. A. and S. K. M conducted the pathogen studies. J. S., H. Z. and V. B. conducted the spectroscopic studies. S. K. M. and A. A. wrote the manuscript draft. All authors contributed to the manuscript writing and reviewing. A. A. and V. B. finalized the manuscript.

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## Conflict of Interests

The authors declare no conflict of interest

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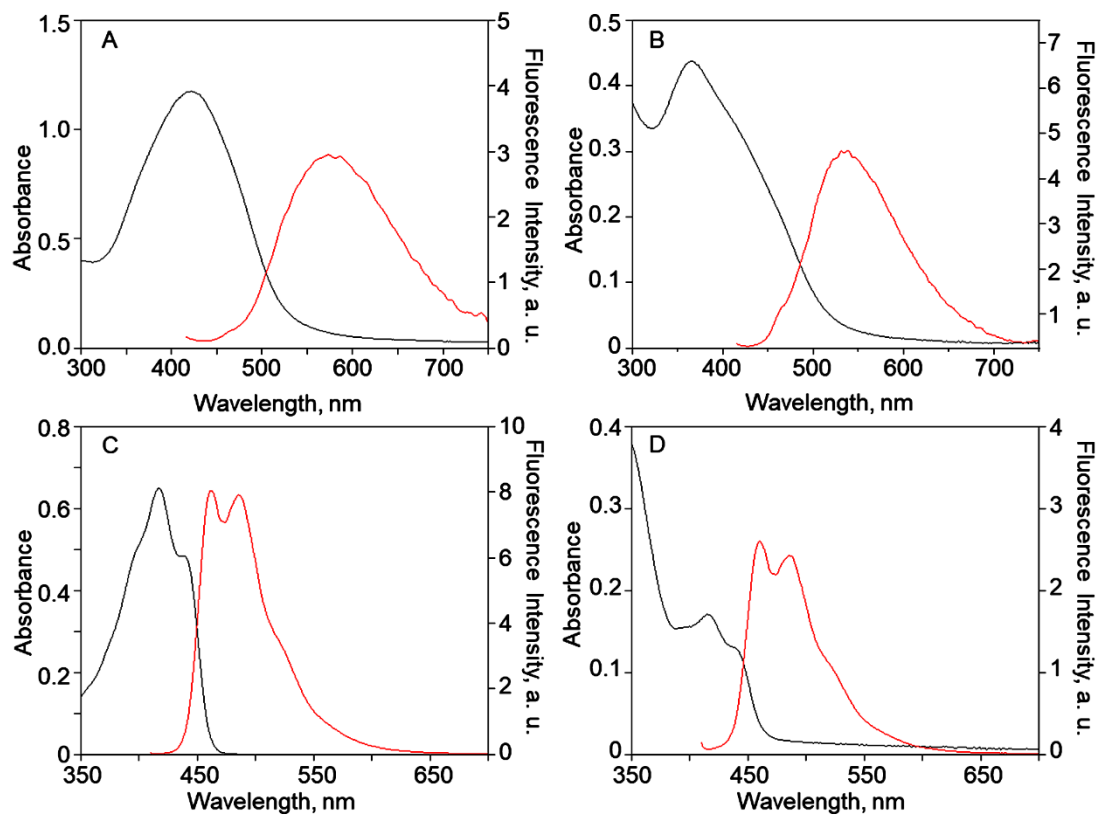
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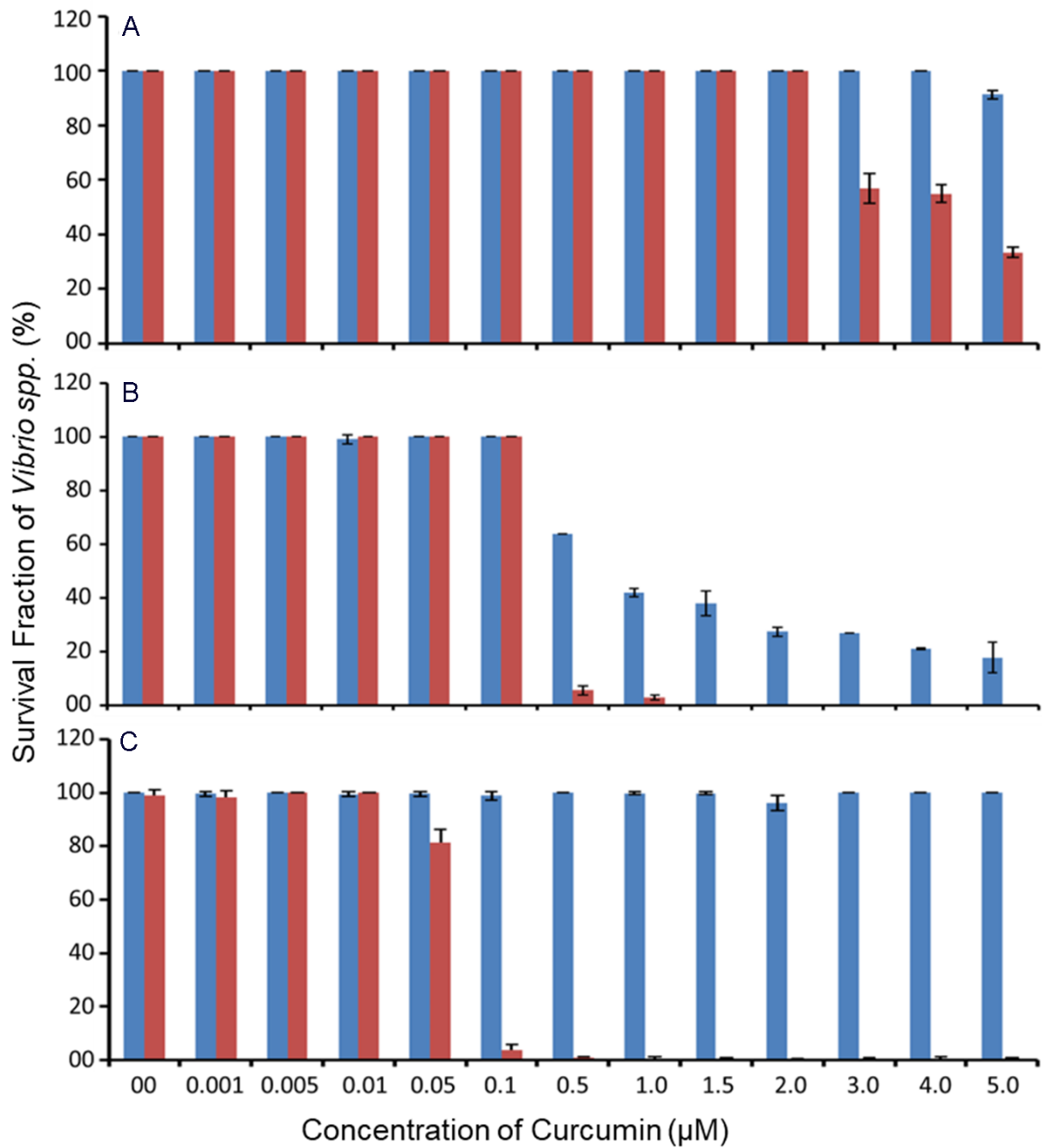
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**Fig 1. Optical properties of curcumin:** Absorption (black line) and fluorescence (red line) spectra of a curcumin solution in (A, B) PBS (15  $\mu\text{M}$ ) and (C, D) toluene (10  $\mu\text{M}$ ) maintained under dark (A, C) or (B, D) exposed to a 405 nm (10  $\text{mW cm}^{-2}$ ) LED for 60 min.

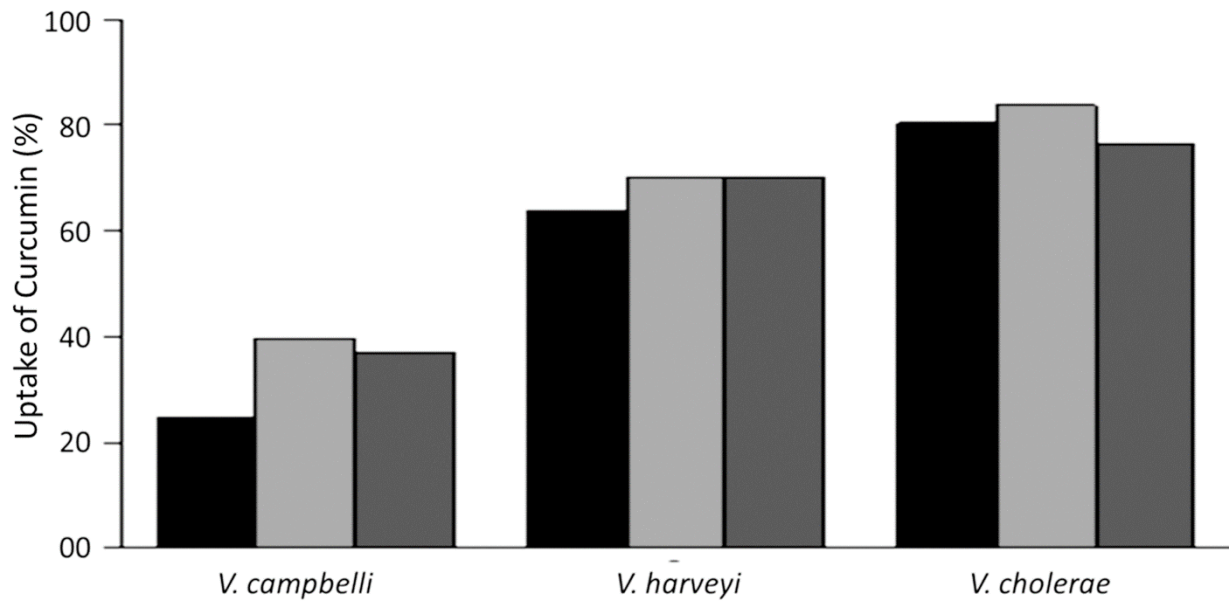




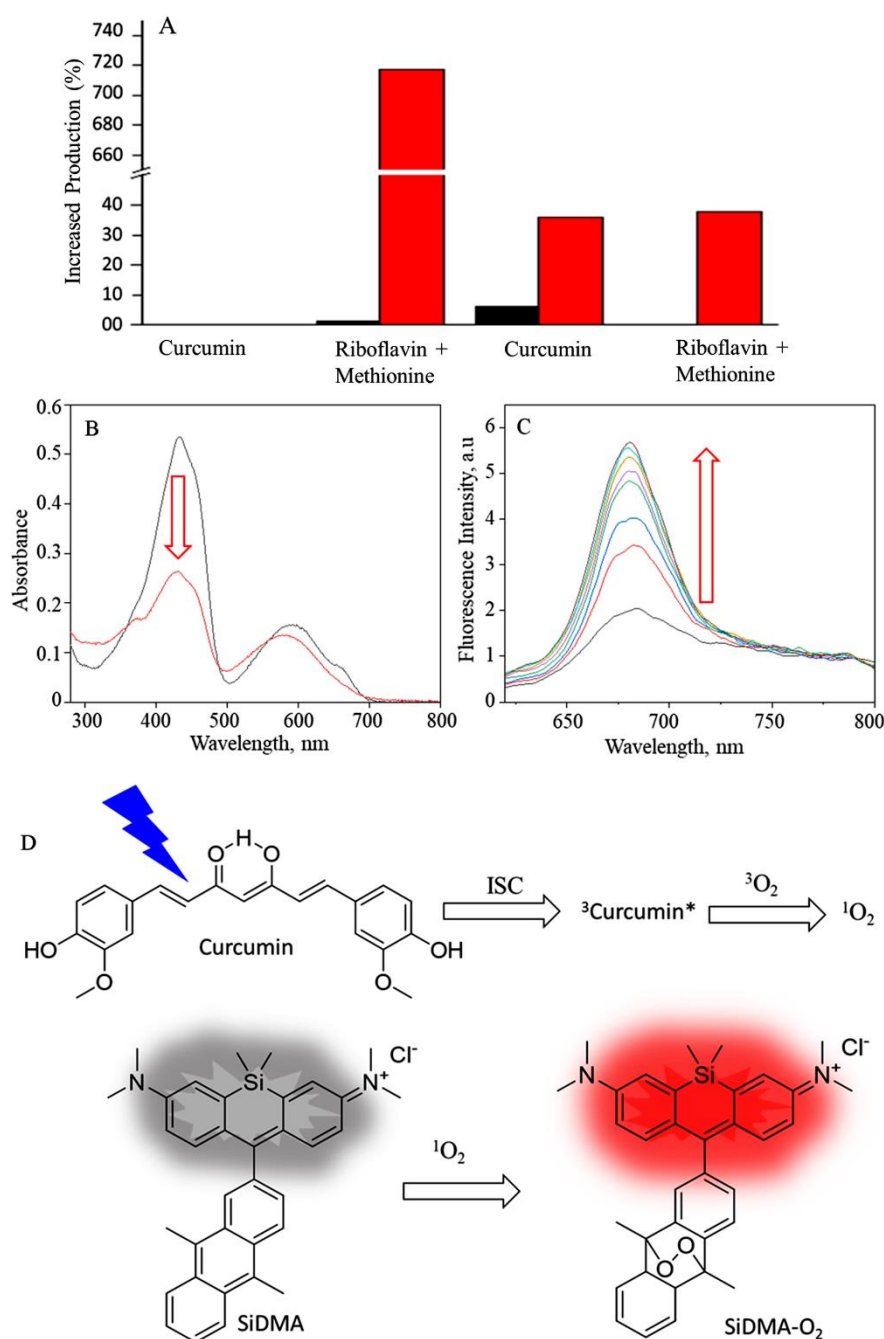
**Fig 2. Bacterial survival efficiency:** Fractions of (A) *V. campbelli*, (B) *V. cholerae*, and (C) *V. harveyi* survived after exposure to different concentrations of curcumin without (blue) and with (red) photoirradiation (11 mW cm<sup>-2</sup>, 450 nm)



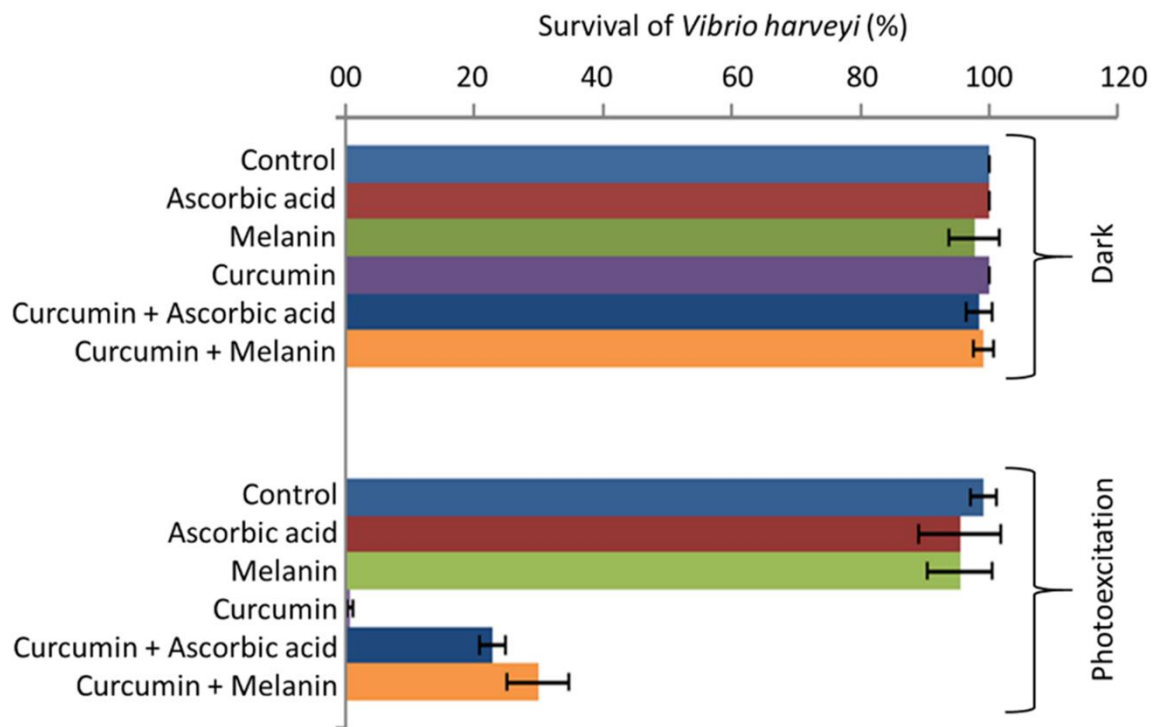
**Fig 3. Curcumin uptake by *Vibrio* Spp.:** Percentage uptake of curcumin with increasing concentrations [10 (black), 50 (grey) and 100  $\mu$ M (dark grey)].



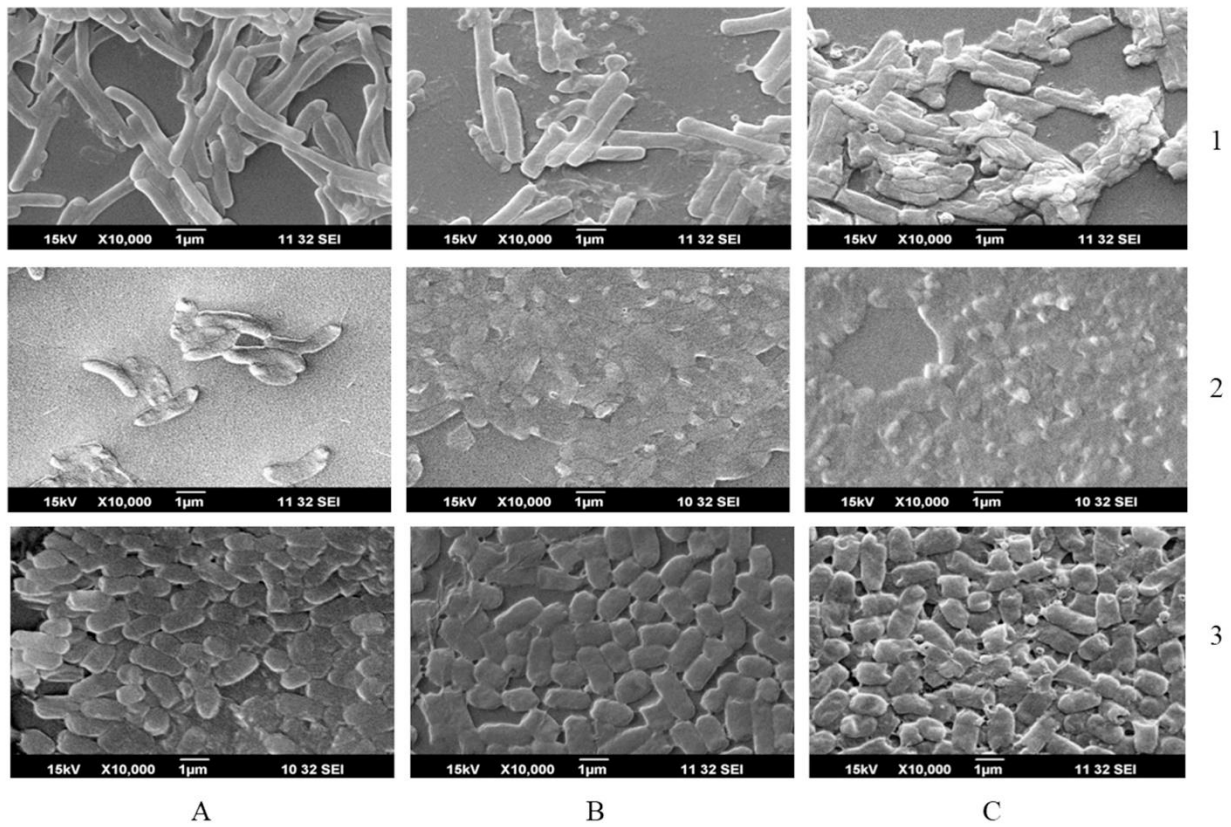
**Fig 4. ROS estimation:** (A) Production of superoxide radicals and singlet oxygen by curcumin under dark (black) and photoirradiation (red). (B) Absorption and (C) fluorescence spectra of SiDMA (1  $\mu\text{M}$ ) in DMSO, in the presence of curcumin (10  $\mu\text{M}$ ) and under 405 nm (10  $\text{mW cm}^{-2}$ ) LED irradiation. The absorption spectra were obtained 15 min before and after the irradiation. The emission spectra were recorded before and every 2 min after the irradiation. (D) A scheme of singlet oxygen generation and detection.



**Fig 5. Antioxidants for PACT survival:** Improved survival of *Vibrio harveyi* in the presence of antioxidants, melanin, and ascorbic acid. The cells were exposed to curcumin under dark and photoexcitation in the presence of antioxidants.

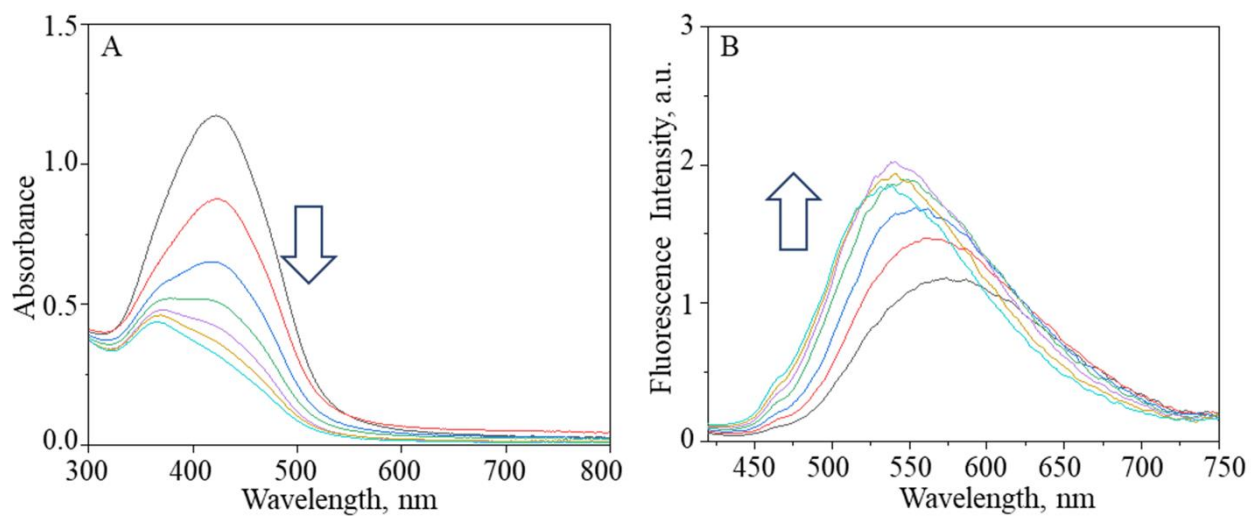


**Fig 6. SEM images of bacteria:** The images of (1) *V. harveyi*, (2) *V. cholerae*, and (3) *V. campbellii* (A) without, and (B, C) with curcumin under (B) dark and (C) 460 nm LED light.

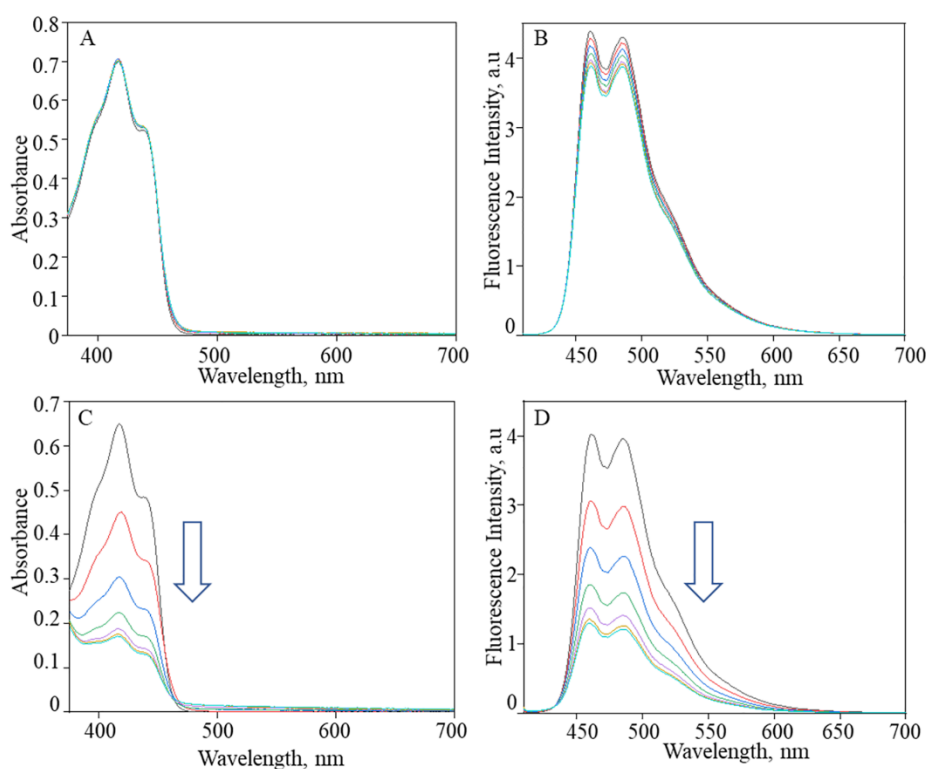


## Supplementary data

**Fig. S1.** (A) Absorption and (B) fluorescence spectra of curcumin solutions (15  $\mu\text{M}$ ) in PBS and under 0 to 60 min 405 nm ( $10 \text{ mW cm}^{-2}$ ) laser irradiation and recorded at 10 min intervals.



**Fig. S2.** (A, B) Absorption and (B) fluorescence spectra of curcumin solutions (10  $\mu\text{M}$ ) in toluene and under (A, B) dark and (C, D) under 405 nm ( $10 \text{ mW cm}^{-2}$ ) laser irradiation for 60 min and recorded at 10 min intervals.



**Fig. S3.** The structures of (A) 9, 10-anthracenediyl-bis(methylene)dimalonic acid (ADMA), and (B) 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (INT).

