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Photocatalytic Degradation Of Methylene Blue And Eosin Yellow And Antioxidant Properties Of Carbon Quantum Dots (Cqds) Derived From *Waltheria Indica*

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Abstract:
This study investigates the synthesis and multifunctional properties of Carbon Quantum Dots (CQDs) derived from *Waltheria indica* leaves using a microwave-assisted method. The CQDs exhibited notable optical properties, including UV-visible absorption peaks at 260-350 nm and excitation-dependent fluorescence emission. Fourier Transform Infrared Spectroscopy (FTIR) revealed the presence of -O-H, C=O, and -C≡C- functional groups. High Resolution Scanning Transmission Electron Microscopy (HRSTEM) confirmed a quasi-spherical morphology with a size distribution of 1.5-5.0 nm. The CQDs demonstrated significant antioxidant activity in the DPPH assay, with an IC_{50} value of 28.86 μg mL^{-1}. Photocatalytic degradation experiments showed that the CQDs effectively degraded methylene blue (MB) and eosin yellow (EY) under visible light, with degradation efficiencies increasing with higher CQD concentrations and longer exposure times. The study concludes that *Waltheria indica*-derived CQDs hold promise for environmental and biomedical applications due to their strong antioxidant and photocatalytic properties.

Keywords: *Waltheria indica*, Methylene Blue, Eosin Yellow, Carbon quantum dots.

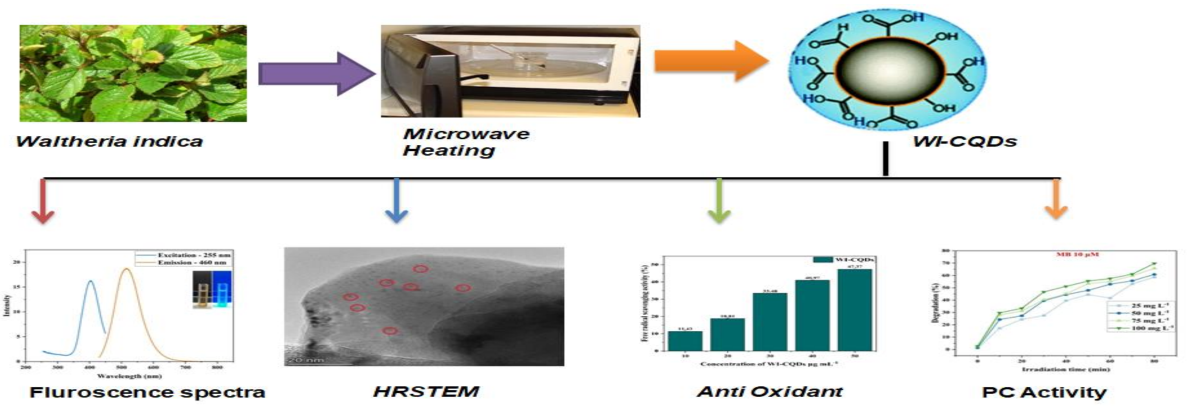
INTRODUCTION

Waltheria indica, a widely distributed medicinal plant, is commonly referred to as sleepy morning and is found in tropical places across the globe. The plant possesses bitter, refrigerant, and astringent characteristics, making it suitable for eliminating moist heat, toxins, and cooling the blood. Native populations in several places of the world also use it to treat various pathological diseases. The plant extract contains several nutritious elements such as caffeic acid, flavonoids, alkaloids, sugar, and tannins, which play a role in the plant's medicinal applications. This plant phytocompounds are potential applications in treating cataracts, diabetes, asthma, anaemia, as well as its potential as an aphrodisiac and anti-cancer agent (C and M, 2021; Zongo et al., 2014).

Currently, there is significant focus on the synthesis of Carbon Quantum Dots (CQDs) for their multifunctional uses. CQDs are crystalline nanoparticles that have a quasi-spherical form and are zero-dimensional. They have extremely small diameters, measuring less than 10 nm, and display the quantum confinement effect (Ashok Kumar et al., 2023). CQDs primarily consist of a sp2 conjugated core and contain oxygen in various forms, represented by several functional groups such as ether, carboxyl, epoxy, hydroxyl, and aldehyde groups. Recently, there has been a strong preference for green synthesis approaches in the production of CQDs, as they offer several advantages over chemical procedures (Ding et al., 2017). These advantages include less chemical exposure, straightforward synthesis protocols, low cost, sustainability, non-toxicity, eco-friendliness, and cost-effectiveness (Dehvari et al., 2019). Remarkably, the raw materials for green synthesis are extremely plentiful on Earth in the form of plants. The many components of plants, including as the root, stem, leaf, fruit, flower, and seed, have been utilised in the production of green CQDs (Wareing et al., 2021). These precursors are cost-effective, less hazardous, widely available, and eco-friendly in comparison to alternative options. Plant precursor-based synthesis eliminates the need for additional reactants for doping, surface passivation, or post modification. This is because carbohydrates, proteins, amino acids, and other biomolecules naturally present in plants offer enough components for the desired surface functionality of CQDs (George et al., 2023).

Methylene blue (MB) and Eosin yellow (EY) are synthetic dyes known for their toxicity to aquatic life and potential human health hazards. Prolonged exposure can lead to adverse health effects, and their persistence in the environment poses significant ecological risks. MB and EY are resistant to conventional wastewater treatment processes due to their stable chemical structures. This resistance complicates their removal from contaminated water, necessitating advanced treatment methods like photocatalysis, which can be resource intensive and costly (Singh et al., 2022). The disadvantages in treating pollutant dyes using conventional methods further requires sustainable alternative methods. Recent studies highlight the potential of plant-derived CQDs for photocatalytic degradation of dyes such as methylene blue (MB) and eosin yellow (EY). CQDs synthesized from biomass sources like coconut husk and onion have shown effective degradation under visible light. These CQDs often exhibit enhanced photocatalytic activity when doped with elements like nitrogen or combined with other materials, achieving significant removal efficiencies of MB and other dyes. Such eco-friendly and efficient approaches offer promising solutions for wastewater treatment (Chávez-garcía et al., 2024; Rani et al., 2023).

Thus, the objectives of the present study are (i) To synthesize the CQDs from *Waltheria indica* leaves using microwave assisted method (ii) To determine the optical and morphological properties of CQDs using various analytical techniques (iii) To evaluate *in vitro* antioxidant properties of CQDs and (iv) To determine the photocatalytic degradation properties of CQDs on MB and EY



Schematic Representation of WI-CQDs

Methodology:

Microwaves assisted synthesis of Carbon Quantum Dots (CQDs):

The fresh leaves of *Waltheria indica* (WI) were collected from Rajamahendravaram, Andhra Pradesh, India and washed with running tap water followed by distilled water. The shade drying was performed to remove the moisture and dried leaves were pulverized into fine powder using electronic blender. 2 grams of WI powder was dispersed in double distilled water and kept on stirrer at 100°C for 1hr to obtain homogeneous distribution of particles. This solution was further exposed to conventional microwave oven at 640W irradiation for 10 minutes with interval of 30 seconds. Then, the reaction mixture was filtered using Muslin cloth and Whatman No.1 filter paper and centrifuged at 5000 rpm for 5 minutes. The CQDs were purified by filtering the supernatant with 0.22 µ PVDF membrane syringe filters (George et al., 2023).

Optical and Morphological properties of CQDs:

The optical and morphological properties of WI-CQDs were evaluated using various analytical techniques. The WI-CQDs were diluted with distilled water in 1:10 dilution ratio and absorption spectrum were recorded using UV-Visible spectrophotometer (Shimadzu 2600) from 200-800 nm wavelength region. The functional groups associated with WI-CQDs were identified using Fourier Transform Infrared Spectroscopy (FTIR) (Bruker Alpha II) from 400-4000 cm^{-1} wavenumber. The excitation and emission wavelengths of WI-CQDs were recorded using Fluorescence Spectrophotometer (PERKIN ELMER) from 200-800 nm wavelength region. The CQDs were dried on a thin film to evaluate crystalline or amorphous nature of WI-CQDs using X-ray diffractometer (BRUKER) with Monochromatic $K\alpha 1$ radiation at 2θ angles from 10 to 100°. The morphology and size distribution of WI-CQDS was confirmed using High Resolution Scanning Transmission Electron Microscope (HRSTEM) (Thermo Scientific) with copper grid at 200kV. The obtained raw data from various analytical instruments further analysed using ORIGIN-PRO, XPERT HI-SCORE and Image J Software's.

***In vitro* Antioxidant activity:**

In vitro antioxidant properties of WI-CQDs were evaluated using 2,2-Diphenyl-1-picryhydrazyl (DPPH) assay. 3mM of DPPH was prepared in methanol and absorbance of the reagents was adjusted to 0.600-0.650 at 517 nm. To this reagent, various concentrations of methanol dissolved WI-CQDs (10 to 50 µg mL^{-1}) and incubated in dark for 1 hr. The reaction mixtures were centrifuged at 5000 rpm for 5 min and the reduction of DPPH free radicals was recorded by observing the change in absorbance of each reaction mixture at 517 nm. DPPH with methanol and ascorbic acid were used as control and standard respectively. The percentage (%) of free radical scavenging activity and Inhibitory concentration 50 (IC50) were calculated using equation 1 & 2 respectively.

$$\% \text{ of Activity} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100 \quad \text{Eqs..1.}$$

$$IC_{50} = Y = \frac{Max - Min}{1 + \left(\frac{X}{IC_{50}}\right)^{Hill\ coefficient}} \qquad \text{Eqs..2.}$$

Photocatalytic Degradation of Methylene blue and Eosin yellow:

The Photocatalytic degradation of MB and EY using WI-CQDs was carried out using visible light irradiation. In brief, two different concentrations of MB and EY (10 μM and 20 μM) were prepared in double distilled water. Various concentrations of WI-CQDs (25-100 mg L^{-1}) were added to each concentration of both dyes and incubated in dark for 30 mins to achieve adsorption-desorption equilibrium condition. After incubation, the reaction mixtures were exposed to visible light irradiation (100 W tungsten bulb) for 90 minutes. For every 10 minutes, 2 mL of sample from each reaction mixture was collected and neutralized complexes were removed using centrifugation at 5000 rpm for 5 mins. The degradation of each dye at various concentrations of WI-CQDs was observed by reading absorbance of the supernatant at 664 nm and 517 nm for MB and EY respectively. The % of degradation was calculated using equation 1 and C/C0 curve was plotted to understand the pseudo first order kinetics and rate constants.

RESULT AND DISCUSSION:

The microwave assisted synthesis of CQDs is straightforward technique, quick, clean, expensive and high yield producing method (Singh et al., 2019). This method allows homogeneous heating of precursor molecules and to conduct in open vessel (Hu and Gao, 2020). The WI-CQDs in this study were synthesized using microwave assisted method and evaluated their optical and morphological properties.

Absorbance of CQDs:

The WI-CQDs exhibited absorbance peaks between 260 and 350 nm and a tail extending towards the visible region, which is a common characteristic of CQDs depicted in Fig 1. These absorbance peaks are attributed to π-π* and n-π* transitions respectively (Choi et al., 2020).

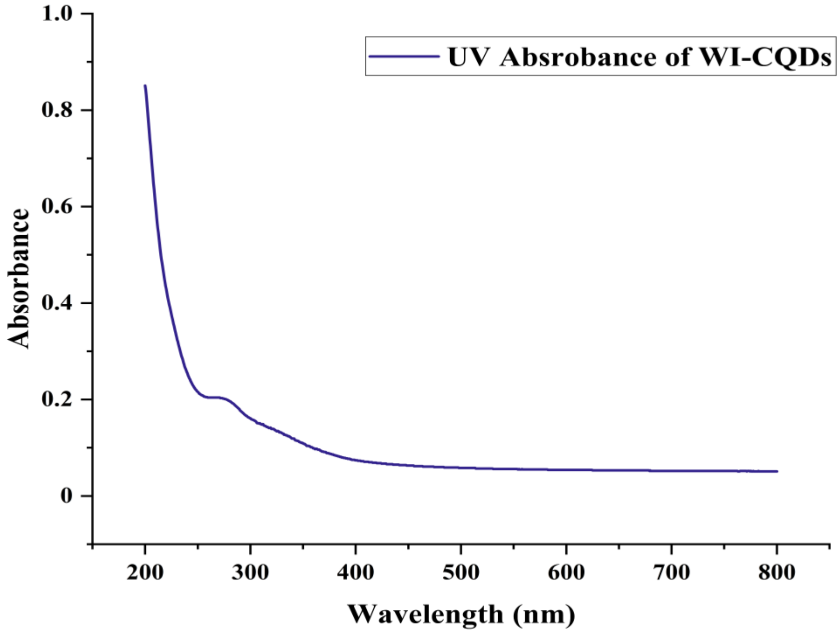


Figure 1: UV Absorbance of WI-CQDs

Surface Functional Groups:

The FTIR spectrum of WI-CQDs depicted in Fig 2 revealed two major vibrational signals at 3344 cm^{-1} and 1632 cm^{-1} which are attributed to O-H/N-H and C=O stretching's respectively. The minor vibrational signal at 2109 cm^{-1} corresponds to weak stretching of C≡C bond. The prevalence of O-H bonding reveals hydrophilic nature of CQDs and possess ample interaction sites which are highly significant in various biological assays (Korram et al., 2023).

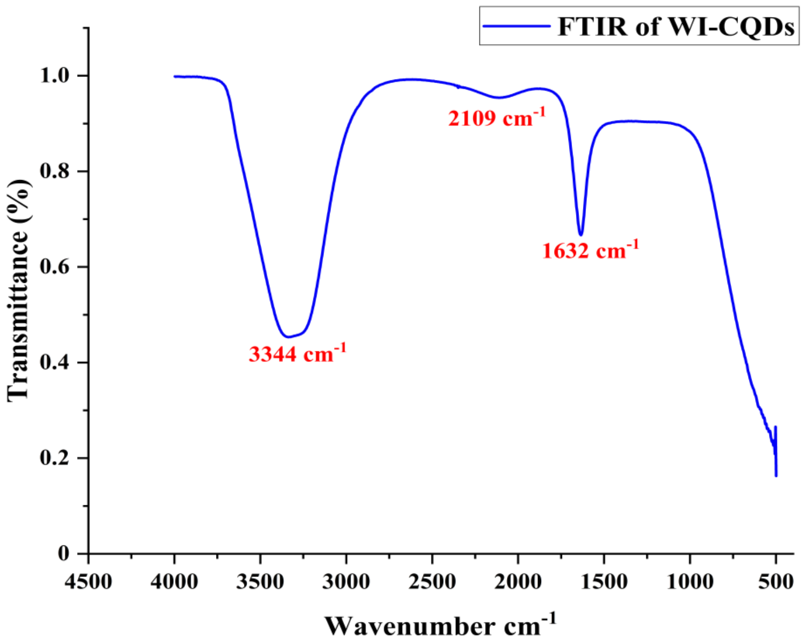


Figure 2: FTIR spectra of WI-CQDs.

Photoluminescence Properties:

The excitation dependent fluorescence emission maximum was observed for WI-CQDs at λ_{max} = 460 nm and the excitation was reported from λ_{max} = 255 nm depicted in fig.3. The application of CQDs is often limited by the dependence of emission wavelength on excitation. This requires the use of multiple excitation sources to achieve diverse colours, and the resulting long-wavelength emissions are typically weak. However, the emission of light at a wavelength that is not affected by excitation can make up for this lack. Various techniques have been suggested to explain the lack of dependence on wavelength of CQDs. These include the size distribution of carbon dots, the arrangement of different emitting traps, the existence of oxygen-containing groups, the formation of various polyaromatic pyrophores through pyrolysis in the carbogenic centre, the presence of free zig-zag sites, and the occurrence of edge defects. The independence of wavelength could also be attributed to the existence of a highly centralised particle size distribution. The microwave-assisted approach for the synthesis of CQDs offers several significant benefits, including the ability to synthesise CQDs in uniform size (Chandra et al., 2013; Du et al., 2016; He et al., 2018).

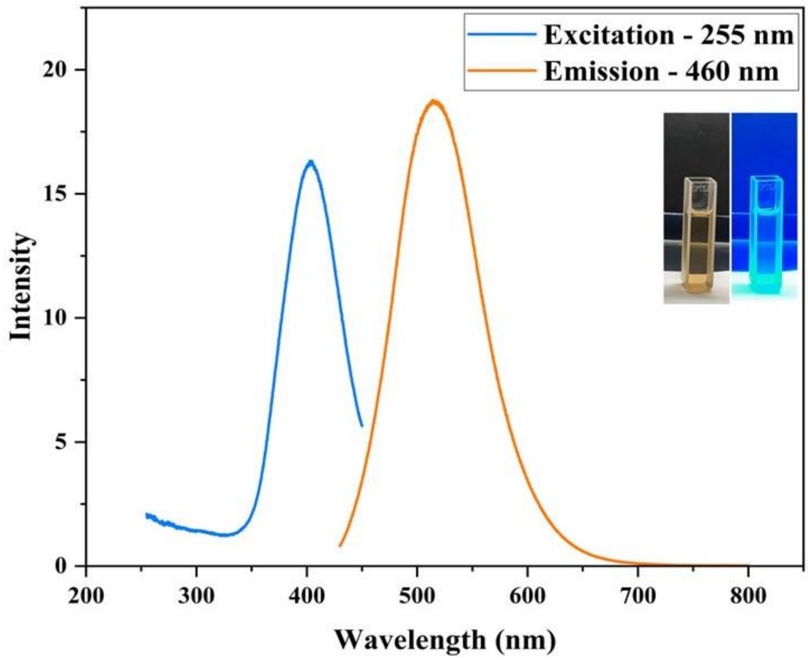


Figure 3: Photoluminescence spectra of WI-CQDs

Phase purity:

The thin film coated WI-CQDs solution was subjected to X-ray diffraction showed a characteristic intense peak at 2θ = 28.26 attributes to lattice plane (0 0 2) which confirmed formation of CQDs with amorphous nature and partial graphitic structure compared with standard ICDD data No. 01-089-8490 (Fig 4). It is vital to emphasise that XRD is not appropriate for characterising amorphous CQDs, as its main purpose is to determine the key characteristics of materials with a crystalline structure. The existence of rich functional groups and the graphitic nature of CQDs are indicated by other minor peaks.

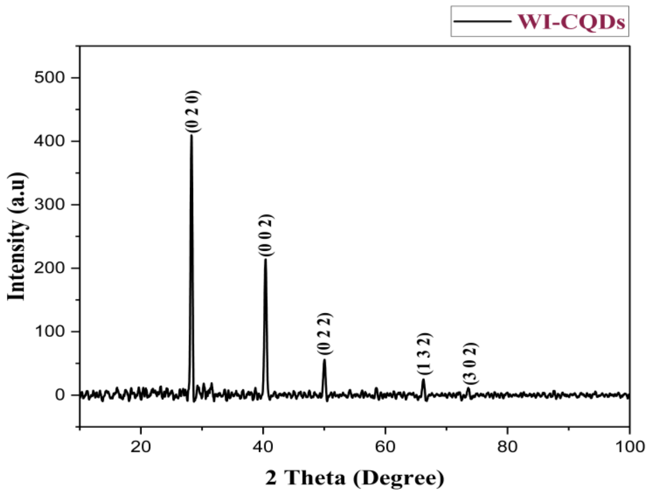


Figure 4: X-ray diffractogram of WI-CQDs.

Morphology and Size distribution:

The HRSTEM micrographs depicted (Fig 5(a)) showed quasi-spherical morphology of WI-CQDs and the size distribution ranges from 1.5 - 5.0 nm with an average particle size diameter 3.40 nm (Fig 5(b)). The EDAX spectrum confirmed the carbon with intense peak at 0.5 keV and presence of O, N, S and P represents the rich surface functional groups (Fig 5(c)).

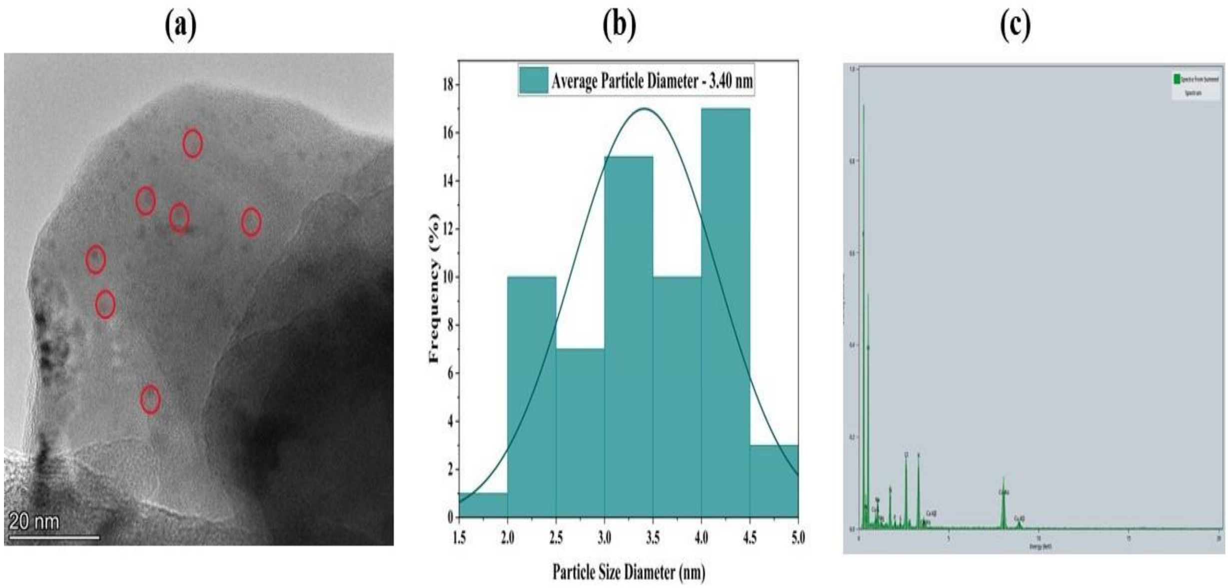


Figure 5: HRSTEM analysis of WI-CQDs (a) HRSTEM micrograph (b) Particles size diameter distribution and (c) EDAX spectrum.

***In vitro* antioxidant activity:**

The DPPH assay was carried out to determine the *in vitro* antioxidant properties of WI-CQDs. DPPH is a nitrogen-based radical that shows absorption at 517 nm. When it is exposed to a potent antioxidant molecule, the DPPH changes from purple to colourless due to the reduction process. The extent of this colour change is influenced by the concentration of the antioxidant agent (Chedea and Pop, 2019). In the present study, the WI-CQDs exhibited 11.43 to 47.37 % of scavenging activity at concentrations of 10-50 µg mL^{-1}. The calculated IC50 value of WI-CQDs was

28.86 μg mL^{-1} represents the amount of CQDs required to scavenge the 50% of free radicals in 1mL of solution.

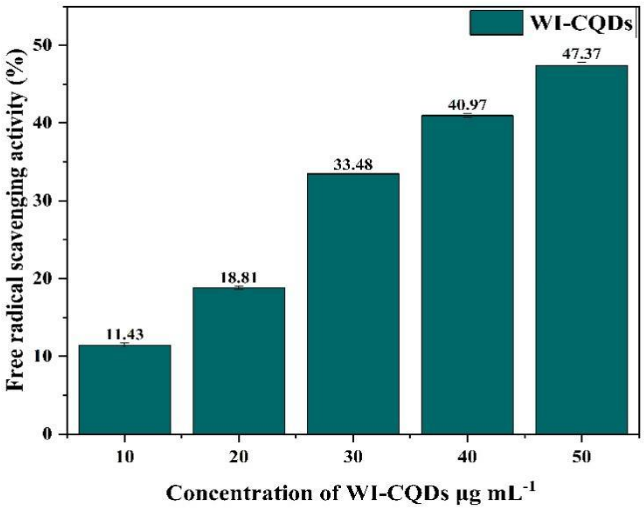


Figure 6: *In vitro* antioxidant activity of WI-CQDs.

Photocatalytic Degradation of MB and EY:

In the present study, two different concentrations of (10 μM and 20 μM) of MB and EY dyes were exposed to four different concentrations of WI-CQDs (25-100 mg L^{-1}) with light irradiation using 100 W tungsten bulb. The degradation efficiency was measured by observing the changes in absorption spectra of two dyes with regular time intervals (0 -80 min). The characteristic absorption band for MB and EY dyes can be observed at 664 nm and 517 nm respectively. The exposure of photocatalyst (WI-CQDs) to the light irradiation result in occurrence of various photochemical reactions on its surface ultimately leads to efficient dye degradation process. The Figure 7-10 represents the dye degradation efficiencies (%) and pseudo first order kinetics of WI-CQDs at various concentrations. Table 1 represents the rate constants of WI-CQDs at each concentration for each dye concentration.

Effect of Time

The photocatalytic degradation of methylene blue (MB) and eosin yellow (EY) using WI-CQDs under visible light irradiation demonstrated significant time-dependent behaviour. For both concentrations of methylene blue (10 μM and 20 μM), the maximum degradation efficiency was achieved at 80 minutes. Similarly, for eosin yellow at both concentrations, the maximum degradation efficiency was reached at 70 minutes. This indicates that the degradation process is highly dependent on the exposure time to visible light, with the optimal degradation times varying slightly between the two dyes.

Effect of Dye and Catalyst Concentration:

The photocatalytic degradation of methylene blue (MB) and eosin yellow (EY) using WI-CQDs showed varying efficiencies at different dye and catalyst concentrations. For methylene blue at a concentration of 10 μM, the degradation percentages were 58.62%, 60.82%, 65.72%, and 69.72% for CQD concentrations of 25, 50, 75, and 100 mg L^{-1}, respectively. At a higher concentration of 20 μM MB, the degradation percentages were slightly lower, at 55.04%, 56.13%, 57.22%, and

59.22% for the same CQD concentrations. For eosin yellow at 10 μM, the degradation efficiencies were 43.37%, 44.32%, 48.85%, and 53.83% for CQD concentrations of 25, 50, 75, and 100 mg L^{-1}. At 20 μM EY, the degradation percentages were considerably lower, at 28.64%, 29.01%, 29.38%, and 29.55% for the same CQD concentrations. These results indicate that higher concentrations of WI-CQDs lead to increased degradation efficiencies for both dyes, with methylene blue showing higher degradation rates compared to eosin yellow, and lower dye concentrations generally resulting in higher degradation percentages.

Pseudo First Order Kinetics and Rate Constants

The rate constants for the degradation of MB and EY were determined using pseudo first order kinetics, which is an effective method for understanding the photocatalytic activity of CQDs. The rate constants were calculated by plotting the slope curve from the C/C0 values, where C is the concentration of the dye at time t, and C0 is the initial concentration. The rate constants for each concentration of WI-CQDs are summarized in Table 1.

The rate constants indicate that the photocatalytic activity increases with the concentration of WI-CQDs, reflecting higher degradation efficiency. This trend is consistent with the hypothesis that higher catalyst concentrations provide more active sites for the generation of reactive oxygen species (ROS), which are crucial for dye degradation.

Pseudo first order kinetics is significant in photocatalytic studies as it simplifies the analysis of complex reactions. It assumes that the concentration of one reactant (typically the dye) is much smaller than that of the catalyst, making its concentration effectively constant. The rate constant derived from this model is a direct indicator of the catalyst's efficiency (Gao et al., 2022). Higher rate constants denote more efficient photocatalytic activity, as observed with increasing WI-CQD concentrations in our study.

Mechanism of Photocatalytic Degradation

The mechanism of photocatalytic degradation using plant-derived CQDs involves the absorption of visible light, leading to the excitation of electrons from the valence band to the conduction band, creating electron-hole pairs. These electron-hole pairs interact with water and oxygen molecules to generate ROS such as hydroxyl radicals (•OH) and superoxide anions (O2•−). These ROS are highly reactive and can break down the dye molecules into less harmful substances. Specifically, for methylene blue and eosin yellow, the ROS attack the chromophore structures, leading to their degradation and decolorization (Akbar et al., 2021; Wen et al., 2023).

Correlation Between Oxidant and Antioxidant Properties of CQDs

CQDs possess both oxidant and antioxidant properties, which play a crucial role in their photocatalytic activity. The oxidant properties, primarily due to the generation of ROS, facilitate the degradation of dyes. Conversely, the antioxidant properties can scavenge excess ROS, preventing potential damage to the CQDs themselves and enhancing their stability. This dual functionality is particularly beneficial in photocatalytic applications, as it ensures a balance between effective degradation and prolonged catalyst lifespan. Studies have shown that CQDs can modulate ROS levels, which is essential for maintaining efficient photocatalytic performance (Innocenzi and Stagi, 2023).

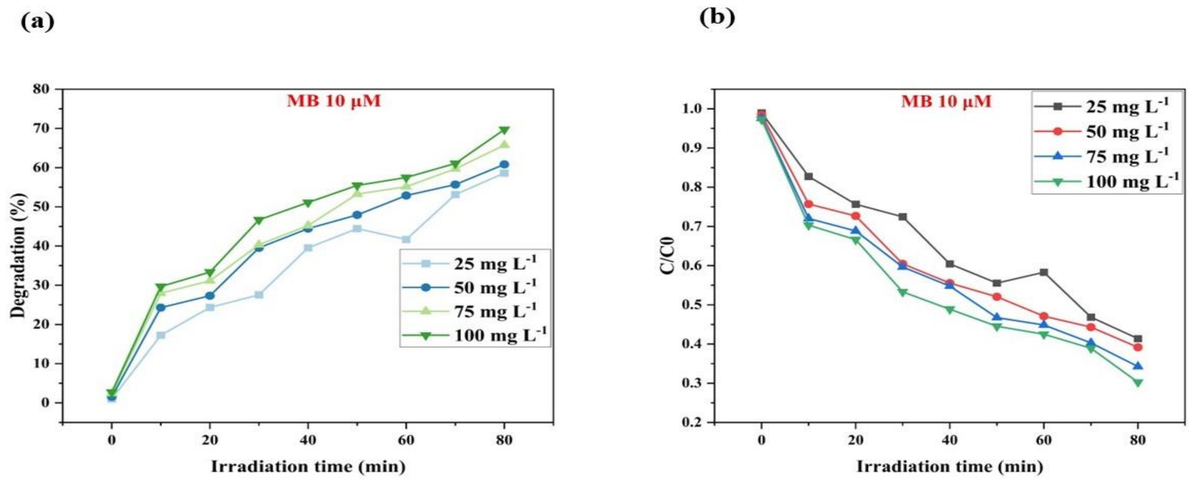


Figure 7: Photocatalytic degradation of Methylene blue (10 μM) (a) Dye degradation (%) at various concentrations of WI-CQDs and (b) Pseudo First Order Kinetics (C/C0 plot).

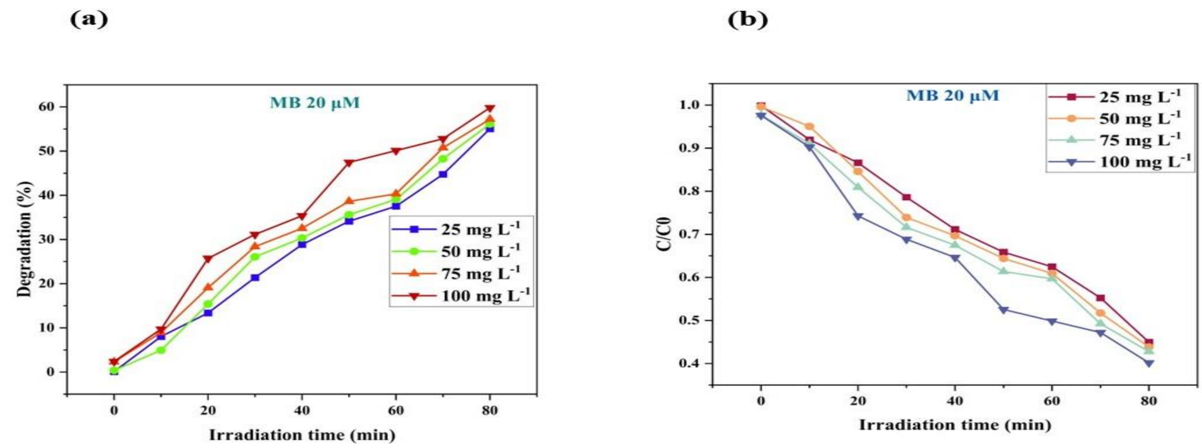


Figure 8: Photocatalytic degradation of Methylene blue (20 μM) (a) Dye degradation (%) at various concentrations of WI-CQDs and (b) Pseudo First Order Kinetics (C/C0 plot).

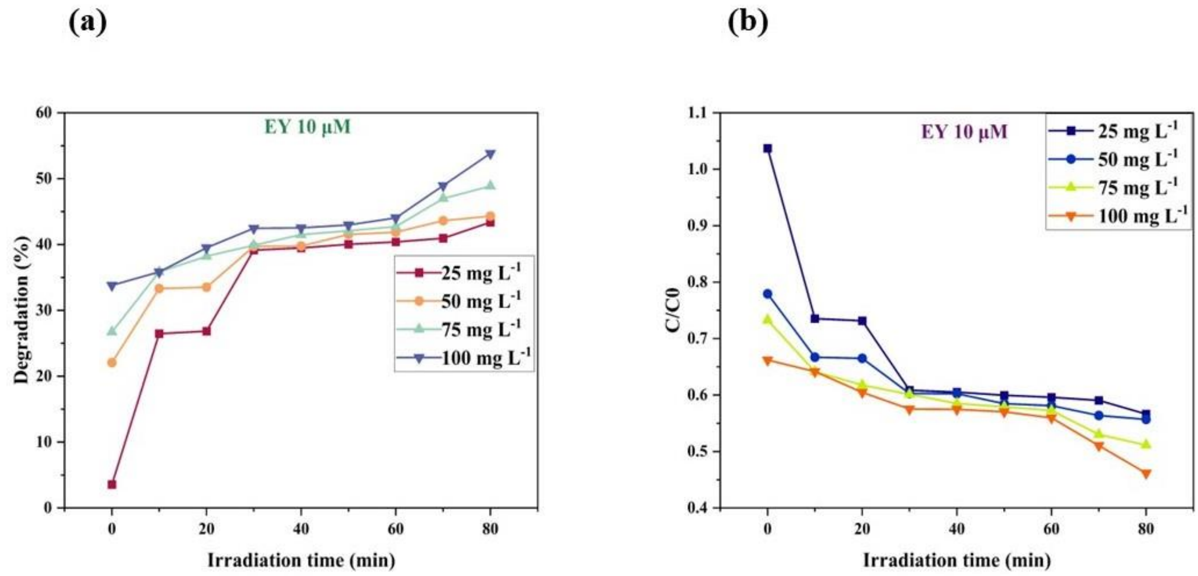


Figure 9: Photocatalytic degradation of Eosin Yellow (10 μM) (a) Dye degradation (%) at various concentrations of WI-CQDs and (b) Pseudo First Order Kinetics (C/C0 plot).

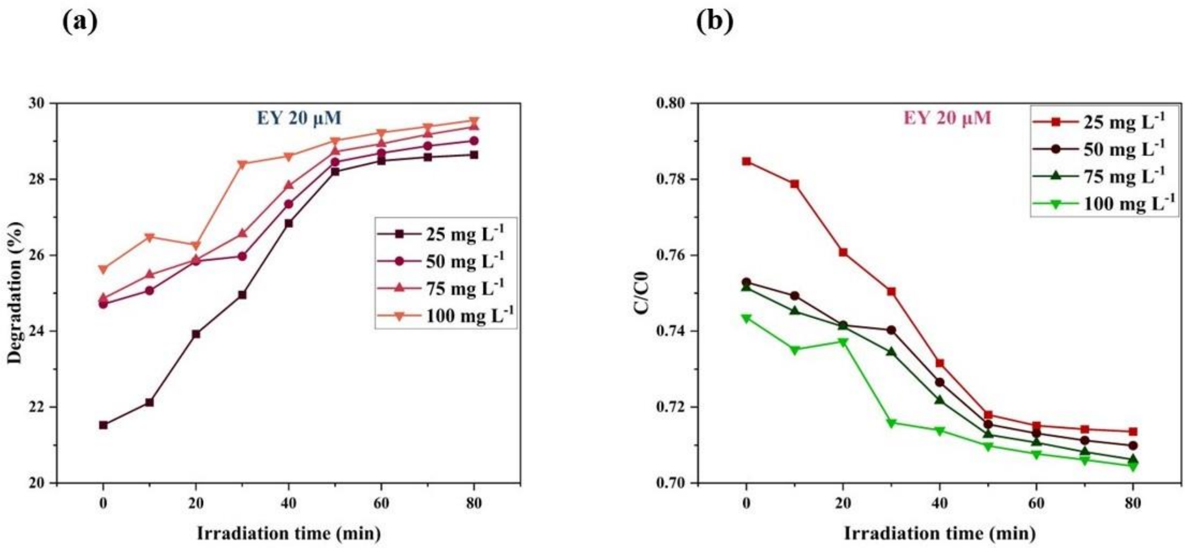


Figure 10: Photocatalytic degradation of Eosin Yellow (20 μM) (a) Dye degradation (%) at various concentrations of WI-CQDs and (b) Pseudo First Order Kinetics (C/C0 plot).

Table 1: Rate constant of WI-CQDs at various concentrations with different concentrations of dyes.

Concentration of WI-CQDs	Rate Constant min^{-1}			
	MB 10 μM	MB 20 μM	EY 10 μM	EY 20 μM
25 $mg\ L^{-1}$	0.0099	0.00926	0.00398	0.00135
50 $mg\ L^{-1}$	0.0105	0.00983	0.00358	0.00084
75 $mg\ L^{-1}$	0.01171	0.00985	0.00366	0.00086
100 $mg\ L^{-1}$	0.0125	0.01094	0.00382	0.00071

Conclusion:

This study successfully synthesized Carbon Quantum Dots (CQDs) from *Waltheria indica* leaves using a microwave-assisted method, showcasing their potential as effective multifunctional materials. The CQDs exhibited remarkable antioxidant properties, with a significant IC50 value in the DPPH assay, indicating their strong free radical scavenging abilities. Additionally, the CQDs demonstrated efficient photocatalytic degradation of methylene blue and eosin yellow dyes under visible light irradiation. The degradation efficiencies were notably dependent on CQD concentration and exposure time, highlighting their potential for environmental remediation applications. The findings underscore the promise of *Waltheria indica*-derived CQDs in addressing pollution through the degradation of harmful dyes, offering an environmentally friendly solution. Moreover, the presence of functional groups identified through FTIR analysis and the nanoscale size confirmed by HRSTEM analysis contribute to the unique properties of these CQDs, making them suitable for various applications. The study suggests a need for further research to optimize the synthesis process, explore mechanistic pathways, and test the CQDs in real-world environmental conditions. Evaluating their toxicity and biocompatibility is also crucial to ensure safety for potential biomedical uses. By addressing these areas, future research can enhance the practical application of these CQDs in environmental and biomedical fields.

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Data Availability Correspondence and requests for materials should be addressed to BN.

Declarations

Competing interests The authors declare no competing interests.

Conflict of Interest The authors declare no competing interests.

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Research Involving Humans and Animals Statement The article does not contain any studies involving human participants and animals performed by any of the authors.

Informed Consent Not Applicable

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