Microwave-Assisted Green Synthesis of Carbon Quantum Dots Derived from *Calotropis Gigantea* as a Fluorescent Probe for Bioimaging

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Abstract
An eco-friendly, cost-effective, and convenient approach for synthesizing biocompatible fluorescent carbon quantum dots (CQDs) from the leaf extract of the medicinal plant *Calotropis gigantea*, commonly known as crown flower, has been demonstrated in this work. Fluorescence quantum yields of up to 4.24 percent were observed in as-synthesized CQDs. The size distribution of the as-synthesized CQDs varied from 2.7 to 10.4 nm, with a significant proportion of sp² and sp³ carbon groups verified by nuclear magnetic resonance analysis. The zeta potential of as-synthesized CQDs was measured to be −13.8 mV, indicating the existence of a negatively charged surface with incipient instability in aqueous suspension. Furthermore, as an alternative to organic or synthetic dyes, the development of simple, inexpensive, and non-destructive fluorescence-based staining agents are highly desired. In this regard, as-synthesized CQDs have shown remarkable fluorescent staining capabilities in this work and might be utilised as a suitable probe for optical and bio-imaging of bacteria, fungi, and plant cells.

Keywords Green synthesis · Fluorescent · Carbon quantum dots · Fluorescent staining · Bioimaging

Introduction
Carbonaceous and carbon-based nanomaterials have received a great deal of interest in recent years because to their enormous potential for practical applications in photocatalysis, optoelectronics, biomedicines, thin film displays, drug delivery, and other engineering and medical disciplines [1–6]. Since their surprising discovery in 2006, carbon quantum dots (CQDs) have demonstrated outstanding photo stability, tiny size, highly regulated photoluminescence, biocompatibility, electrochemiluminescence, and extraordinary multi-photon excitation (up-conversion) capacity [7–11]. Since CQDs may be functionalized with biomolecules and are less toxic and chemically inert in nature, hence, they are suitable carriers for drug delivery, biological imaging, and other applications [12–17]. Semiconducting quantum dots are often synthesized using relatively expensive precursors, however, repeatability remains a challenge [18, 19]. Hence, it is critical to develop and produce a simple, cost-effective, large-scale ecofriendly method/process that makes use of low-cost precursors. In this context, CQDs provide unique and critical features such as excellent biocompatibility, particular biological target, minimum toxicity, and a robust quantum size impact when compared to conventional inorganic QDs, which can be produced utilising low-cost methods [20–24].

In this scenario, natural biomaterial resources are typically preferred over other organic, inorganic, or synthetic resources in the search for an efficient, inexpensive, and environmentally friendly synthesis method of CQDs because they are renewable and biocompatible, and can convert biomass waste into worthy and valuable materials [25]. Natural precursors are also potential chemical alternatives. Besides, natural precursors outperform chemicals in the synthesis of carbon-based nanomaterials due to a variety of advantages, including low cost, nontoxicity, and availability [26, 27]. Several studies on the synthesis of carbon-based nanomaterials from natural precursors, including orange juice, green tea, egg, potatoes, lotus root, pepper, coriander leaves, and others, have recently been reported [28–34].

Green synthesis methods, on the other hand, are considerably more acceptable than physical and chemical procedures.
In comparison to traditional time-consuming hydrothermal procedures, microwave-assisted bottom-up processes may be advantageous for rapid, easy one-pot synthesis, efficient, cost-effective, and energy-saving methods of synthesising high quantum yield carbon quantum dots.

In the meantime the fluorescent nanomaterials have sparked a lot of attention as potential competitors to traditional fluorescent dye probes in recent years, and they've evolved quickly as a result of the growing need for fluorescent probes in chemical sensing, biological labelling, and other sectors. When compared to ordinary fluorescent dyes, fluorescent nanomaterials have the quantum size effect and unique nanomaterial effects, which may overcome many of the latter's disadvantages, such as poor fluorescence intensity, low stability, quick photo bleaching, and so on. Fluorescent nanoparticles such as gold quantum dots, copper or palladium nanoclusters are currently widely employed in a variety of scientific and technical sectors [35–39].

One of the primary goals of this research is to develop a simple and efficient experimental method for low-cost CQD fabrication from the leaf extract of the traditional medicinal plant known as crown flower (Calotropis gigantea) using one-step microwave-assisted synthesis at relatively low temperatures and in a shorter time frame. Calotropis gigantea is a medicinal herb that has been used for thousands of years. Calotropis gigantea is a traditional medicinal plant native to Asian countries that belongs to the “Asclepiadaceae” family and is endowed with immense medicinal properties that are frequently used as Ayurvedic medicine to treat a variety of illnesses such as toothache, earache, sprain, anxiety, pain, epilepsy, diarrhoea, and so on [35].

Meanwhile, the use of fluorescent stains or markers to see prokaryotic (bacteria) and eukaryotic (fungi and plants) cells is rapidly spreading across all disciplines [36]. Microorganisms may be observed directly under a microscope, which is a useful tool in many microbiological studies. This has been seen with protozoa, fungi, injected bacteria, and rhizosphere microorganisms.

Fluorescent dyes have traditionally been used to stain members of many bacterial genera, fungi, and plant cells. Both cationic and anionic dyes (also known as fluorochromes if fluorescent) have been utilised because of their ability to attach to particular biological components of microbial cells. Fluorochromes that are widely employed include acridine orange, ethidium bromide, fluorescein isothiocyanate, and others [40]. However, for a sustainable future, the employment of biocompatible, non-toxic, ecologically friendly, and cost-effective fluorescent materials is important. As a consequence, in this study, we demonstrated the key material and optical properties of CQDs synthesised from Calotropis gigantea leaf extract, as well as a fluorescent staining approach for utilising those CQDs as fluorescent markers for bio-imaging.

### Experimental Section

#### Chemicals and Materials

Fresh Calotropis gigantea (crown flower) leaves were harvested in Bhambol, Yamunanagar, Haryana, India. The chemicals used in this investigation were all of analytical grade. Other materials purchased from a commercial supplier included Whatman filter paper (grade 1), 0.22 μm syringe filter (polytetrafluoroethylene membrane), acetone, and isopropyl alcohol. Fluorescent staining was done with Bacillus subtilis, Escherichia coli, bacteria Aspergillus fungi, and Tradescantia pathacea (Sitara Plant). A Milli-Q system was used to obtain deionized water (18.2 MΩ.cm⁻¹) (Millipore, France).

#### Microwave-assisted Green Synthesis of Carbon Quantum Dots

Fresh Calotropis gigantea (crown flower) leaves were collected and processed for extraction. The leaves were thoroughly rinsed with tap water, then distilled water to remove dust particles, air-dried, and incised into small pieces. In the beginning, 10 gm of fresh crown flower leaves were crushed into a powder in a clean environment and mixed with 100 ml of distilled water. The blended aqueous extract solution was filtered through Whatman qualitative filter paper (grade 1) to remove fibrous impurities before being placed in a 900 W domestic microwave oven [41]. The microwave processing time was meticulously adjusted until brown viscous fluid was obtained. To remove the large agglomerate particles afterwards, it was centrifuged for 15 min at 10,000 rpm. A 0.22-μm syringe filter was then used to filter the supernatant (polytetrafluoroethylene membrane). Finally, the water-suspended CQD solution was kept at 4°C for future use. The complete procedure is depicted graphically in Fig. 1.

#### Apparatus and Characterization

The as-synthesized CQDs were characterised using several characterisation methods. Shimadzu UV-2700 spectrophotometer was used to measure UV–visible absorption spectra. Varian Cary Eclipse fluorescence spectrophotometer was used to record the fluorescence emission spectra. Transmission electron microscopy was used to confirm the average size and morphology of the CQDs (Hitachi, H-7500). The TEM analysis yielded selected area electron diffraction (SAED). Panalytical’s X’Pert Pro was used to generate the powder X-ray diffraction (XRD) pattern. The Fourier transform infrared spectrum (FTIR) was measured on a (PerkinElmer Spectrum 100) in the 500 to 4000 cm⁻¹ range. The
Nano Zeta-Sizer Malvern apparatus was used to determine the zeta potential. The nuclear magnetic resonance (NMR) spectroscopy (FT-NMR) was carried out using an FT-NMR spectrometer (Avance Neo, Bruker). Fluorescence images were captured using a Nikon Eclipse E600 fluorescence microscope.

**Quantum Yield Determination**

As a standard, conventional quinine sulphate (0.1 M H₂SO₄ as solvent; QY = 0.54) was chosen. The slope method was used to determine the QY of CQDs (in water) with reference to quinine sulphate. The curve was obtained by comparing the photoluminescence intensity and absorbance values of the samples to those of the reference (several values less than 0.1 at excitation wavelength). The equation used for QY calculation is [42]:

\[
QY_{QD} = QY_{ref} \cdot \frac{I_{QD}}{I_{ref}} \cdot \frac{A_{ref}}{A_{QD}} \cdot \frac{\eta_{QD}^2}{\eta_{ref}^2}
\]

where \( QY_{ref} \) is the quantum yield of the reference sample, \( \eta \) is the refractive index of the solvent, \( I \) is the integrated fluorescence intensity and \( A \) is the absorbance at the excitation wavelength. The CQDs were diluted in deionized water to achieve the necessary concentrations. A UV–vis spectrophotometer was used to test the absorbance of CQDs at varied concentrations. The emission spectra of particular concentrations of CQDs were measured using a fluorescence spectrometer at an excitation wavelength of 280 nm, and the allied integrated intensity was computed. The QY of CQDs was found to be 4.24%.

It is worth noting that the higher quantum yield of CQDs derived from diverse parts of medicinal/herbal plants is still a source of concern. Table 1 compares the synthesis process, quantum yield, and particle size of CQDs. Because of the diversity of carbon sources, QY of high-temperature pyrolysis synthesis is often lower than that of hydrothermal or microwave-assisted synthesis.

**Cell Culture/ Staining Technique**

The gram-positive and gram-negative bacteria (Escherichia coli, Bacillus subtilis) were streaked on nutrient agar before being inoculated into liquid nutrient broth culture medium and grown at 37 °C with 100 rpm shaking for 18–24 h [53].

**Bacterial Staining**

First, a bacterial smear was stained with fluorescent CQDs (concentration in the range between ~0.1–0.2 mg/ml) and allowed to dry before being heated. The smears were then held in place with a slide rack or a clothes pin. The fluorescent dye was then carefully applied to each smear. Before inspecting the strained slides under a fluorescent microscope, they were allowed to air dry.
Fungal Staining

To begin, a drop of fluorescent CQDs (~0.1–0.2 mg/ml) was put on a clean slide, followed by a tiny tuft of fungal mycelium containing spores. Then, gently tease the material with the two mounted needles. Following that, the slide was viewed using a fluorescence microscope while covered with a cover slip to prevent air bubbles from being trapped in the stain.

Plant Cell Staining

The removal of the leaf epidermis in order to examine the quantity, arrangement, distribution, and structure of stomata is known as peeling. The technique includes using force to shatter the leaf unevenly. This readily separates a little part of the bottom epidermis that is still protruding on the lower surface of the leaf. A lengthy ribbon or strip of lower epidermis is ripped off. Before being viewed under a fluorescence microscope, the lower epidermis is scraped, dyed with fluorescent CQDs (~0.1–0.2 mg/ml), and coated with a coverslip. After that, the coverslip was put on the slide and inspected using a fluorescence microscope.

Fluorescent Stain Screening

Bacterial growth was achieved using nutrient broth medium, which was incubated at room temperature for 24 h (30–37 °C). On separate glass slides, tiny smears of microorganisms were produced first. Allow the smear mixture containing the produced fluorescent stain to dry naturally. Each smear was stained with fluorescent dye for 30 s. After that, the coverslip was put on the slide and inspected using a fluorescence microscope. When the adhesion values stabilised at 30 to 40%, the fluorescent stains were screened using a biological fluorescent stain at an optimised concentration. Microorganisms can be stained at predictable locations across and throughout the contaminated regions using fluorescent dye at effective concentrations, as established by staining procedures. In this study, we focused on developing fluorescent staining methods to detect and locate bacteria and fungus in a variety of samples.

Results and Discussion

Composition, Structure and Morphology Analysis

The size and morphology of as-synthesized CQDs derived from Calotropis gigantea were investigated using high resolution TEM. Figure 2a shows the almost spherical form of carbon dots, while Fig. 2b shows the corresponding histogram, which indicates that the average size of CQDs is 5.7 nm, with a range of 2.7 to 10.4 nm. It is worth noting that CQDs are not evenly distributed; rather, they are attempting to agglomerate due to the absence of any passivating agent. In fact, the measured Zeta potential value was around −13.8 mV, indicating the presence
of a negatively charged surface with incipient instability in water suspension. Furthermore, the broad diffused ring in the selected area electron diffraction (SAED) pattern revealed the as-synthesized CQDs' poor poly-crystalline nature (Fig. 2c).

XRD was used to investigate the crystalline nature of as-synthesized CQDs. X-ray diffraction pattern obtained with PANalytical X’pert Pro MPD powder X-ray diffractometer with Cu Kα (λ = 1.54 Å) radiation confirmed relatively better crystalline nature. Figure 3’s XRD spectrum confirms that the synthesized CQDs are poly-crystalline, with a broad peak around 2θ = 25° due to the presence of amorphous carbonaceous core–shell materials [54, 55].

The observed relatively steep peaks at 28.4°, 40.6°, 50.3°, and 58.8°, however, correspond to the crystal planes (002), (100), (102), and (103), where the first three are graphite (sp²) and the last one is diamond (sp³), which is comparable to carbon [56]. The d-spacing for (002), (100), (102), and (103) planes was calculated to be 0.31, 0.22, 0.18, and 0.16 nm, respectively, which is similar to the graphitic lattice spacing [57].

On the other hand, FTIR data revealed the presence of various oxygen functional groups and linkages in carbon quantum dots (see Fig. 4). The presence of –OH, –C–H, C = O, O–H, and C–O was clearly indicated by stretching frequencies at 3487, 2980, 1745, 1380, and 1078 cm⁻¹, respectively [58, 59]. The presence of these functional groups suggests that the carbon quantum dots that were synthesized have a high water solubility.

Furthermore, nuclear magnetic resonance (NMR) spectroscopy (¹H and ¹³C) of CQDs was utilized to distinguish between sp³-hybridized carbon atoms and sp²-hybridized carbon atoms. The NMR spectra displayed in Fig. 5 demonstrate the presence of four different chemical environments in the four separate areas mentioned below.
The following chemical shift (δ) regions were discovered in the $^1$H NMR spectrum (Fig. 5a): 1–3 ppm (dominated by sp$^3$ C–H protons), 3–6 ppm (associated with protons of hydroxyl, ether, and carbonyl groups), 6–8 ppm (for aromatic or sp$^2$ protons), and 8–10 ppm (related to aldehydic protons) [60]. The $^1$H NMR spectrum in Fig. 5a shows that the first two regions mentioned above are dominated over the last two regions, indicating that sp$^3$-hybridized carbon atoms are dominated over sp$^2$-hybridized carbon atoms. Carbon quantum dots are indeed mixed with sp$^3$ and sp$^2$–hybridized carbon atoms. Meanwhile, the $^{13}$C NMR spectrum revealed a mixture of sp$^3$ and sp$^2$–hybridized carbon atoms (see Fig. 5b). There were four distinct regions found, namely 20–80 ppm (for sp$^3$ carbons as well as carbons attached with hydroxyl groups), 80–100 ppm (for carbons attached with ether linkages), 100–120 ppm (for C=C aromatic or sp$^2$–hybridized carbon atoms), and finally 175–190 ppm (for C=O carbons) [61].

**Absorbance and Luminescence Properties**

The optical absorption peak of as-synthesized carbon quantum dots at a concentration of ~150 μg/ml was observed in the UV region, with a maximum absorption around 280 nm and a tail extending into the visible range (see Fig. 6 for detail). This is due to the usual n–π*transition of the C=O band and the π–π* transition of the conjugated C=C band [62]. Besides, UV–Visible spectrometry may also be used to display quantum or nano dots in order to measure their band gap energy and particle sizes. The cut-off wavelength was initially estimated by intersecting the peak’s tangent line with the wavelength axis.

This wavelength is used to calculate the band gap $E_{g}^{CQD}$ of the CQD [63]:

$$E_{g}^{CQD} = \frac{hc}{\lambda_{edge}}$$  \hspace{1cm} (2)

where $\lambda_{edge} = \lambda_{max}$ wavelength absorbed by the CQD sample, and $c$ is the speed of light. Firstly, a line of best fit was determined for the linear portion near the peak in the spectrum, as shown in inset of Fig. 6.

The equation for best-fitting line is shown, along with an $R^2$ value close to 1 to ensure that this portion is linear. Using this line’s equation, the cut-off wavelength of 404.8 nm and band gap of 3.063 eV were calculated. Secondly, using the following expression derived from the following effective mass model, the particle size can be estimated from the experimental UV–Vis absorption spectrum [63]:

$$E_{g}^{CQD} = E_{g}^{bulk} + \frac{\hbar^2 \pi^2}{2r^2} \left( \frac{1}{m_e} + \frac{1}{m_h} \right)$$

$$- \frac{1.8e^2}{4\pi\epsilon\epsilon_0 r^2} - \frac{0.124e^4}{\hbar^2 (4\pi\epsilon\epsilon_0)^2} \left( \frac{1}{m_e} + \frac{1}{m_h} \right)^{\frac{1}{2}}$$  \hspace{1cm} (3)

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*Fig. 5* (a) $^1$H-NMR and (b) $^{13}$C-NMR spectra of the as-synthesized carbon dots
where $E_{ECD}^g$ = band gap energy of CQD, which will be determined from the UV–Visible absorbance spectrum. $E_{ECD}^{Bulk}$ = band gap energy of the bulk at room temperature, $\hbar =$ Planck’s Constant, $6.625 \times 10^{-34}$ J \cdot s, $r =$ particle radius (m), $m_e =$ mass of a free electron, $9.11 \times 10^{-31}$ kg, $m^*_e =$ (effective mass of a conduction band electron), $m^*_h =$ (effective mass of a valence band hole), $e =$ elementary charge, $1.602 \times 10^{-19}$ C, $\varepsilon_0 =$ $8.854 \times 10^{-12}$ C$^2$N$^{-1}$m$^{-2}$ (permittivity of free space), $\varepsilon =$ is the relative permittivity. The estimated CQD size was around 3.82 nm, which is consistent with the observed HRTEM images.

Meanwhile, carbon dots are distinguished by their emission wavelength and size-dependent fluorescent behavior. Figure 7 depicts the fluorescence spectrum of CQDs. The emission peak ranged from 340–480 nm after excitation with a wavelength of 280 nm. The deconvoluted fluorescent spectrum revealed two distinct broad peaks, with maximum emission peaks occurring at 354 and 441 nm, respectively, indicating multi-color fluorescent emission.

**Development of a Fluorescent Staining Method for Monitoring Bacteria, Fungi and Plant Cells**

The purpose of this research is to find alternative fluorescent stains or markers for bacteria, fungi, and plant cells. One of the most important conditions for any fluorescent stain is that it has very modest impacts on bacterial attachment, feasibility, and metabolic activity while remaining in cells for at least a few weeks in order to be utilised to investigate bacterial transport and monitor bioaugmentation. 5-sulfofluorescein diacetate, sodium salt (SFDA), 5-cyano-2,3-ditolyl-tetrazolium chloride (CTC), fluorescamine and other fluorescent staining agents have previously been reported in the literature [64]. During staining with such chemicals, the manufacturer’s suggested methods, such as suitable carrier solvents, cell suspension buffers, final concentrations, and temperature settings, must all be followed. Besides,
to achieve final concentrations, the compounds must be added as concentrated stocks in carrier solvents containing dimethyl sulfoxide (DMSO) or dimethyl formamide at the appropriate concentrations [64]. The aforementioned lengthier procedures, however, are not required for the new CQD-based fluorescent stain derived from Calotropis gigantea in this work. Despite the fact that the general staining technique was adequate for initial screening, an attempt was made to minimise the quantity of fluorescent stain needed to stain a specific number of cells while simultaneously optimising the overall staining procedure. This was selected primarily to reduce the expense of labelling cells for field-scale research while also allowing the staining procedure’s scaling-up. Various staining combinations were performed such as staining of stationary cultures, staining of log-phase cultures, staining with ambient temperature (32 °C) or cycling temperature ranges from 25 to 37 °C and staining with different fluorescent stain concentrations (10, 50, and 100 μl). Fluorescent staining typically took 2 to 4 h. The staining was assessed qualitatively using a Nikon Eclipse E600 fluorescence microscope equipped with 100 Watt mercury lamp, an
episcopic-fluorescence attachment, an H-III photomicrographic attachment and various color filters.

Fluorescent microscope was used to analyse the bacterial form and cell configurations, as illustrated in Fig. 8a, which displays a rod-shaped Bacillus and a tiny rod of E. coli. Similarly, fungal slides were examined under a fluorescent microscope to determine the type of hyphae, conidiophore, and conidia, as well as their arrangements. The fungal cytoplasm is visible on the slide as a fluorescent colour region containing hyphae, conidiophores, phialides, and conidia surrounded by fluorescent colour (see Fig. 8b). Figure 8c shows the plant cell wall of Tradescantia spathacea, which revealed detailed cell structure and stomata. However, it is worth noting that the high emission quantum yield of carbon quantum dots is still remains a challenge and is required for their usage in bioimaging applications. Furthermore, the defects in carbon quantum dots play an essential role in the fluorescence nature, and bioimaging sensitivity or selectivity must be enhanced.

Conclusions

In conclusion, carbon quantum dots were synthesised from the medicinal plant Calotropis gigantea as a green source in a one-step, simple, eco-friendly, and cost-effective method using a microwave assisted synthesis technique. Following UV-excitation, the as-synthesized carbon dots demonstrated excellent fluorescence intensity, high photostability, and efficient multicolor fluorescent emission. In fact, as-synthesized CQD fluorescence quantum yields (QYs) achieved 4.24 percent. According to HRTEM and XRD studies, CQDs have an average size of less than 10 nm, a near-spherical shape, and are mainly crystalline in nature. Meanwhile, NMR spectra showed the existence of both sp² and sp³ carbon groups in significant amounts. Furthermore, the as-synthesized CQDs were found to be incipiently instable in water suspension, with Zeta potential measurements revealing the presence of a negative surface charge (-13.8 mV). Because of their simplicity, low cost, and green production, CQDs produced from Calotropis gigantea have been standardised as an alternative fluorescent staining agent in biolabeling of bacteria, fungi, and plant cells.

Author Contributions Dr. M. K. Bera contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mrs. N. Sharma and Dr. I. Sharma. All authors read and approved the final manuscript.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

References

1. Peixotode B, Monteiroda O (2021) Carbon quantum dots synthesis from waste and by-products: Perspectives and challenges. Mater Lett 282:128764
2. Ghosh D, Sarkar K, Devi P et al (2021) Current and future perspectives of carbon and graphene quantum dots: From synthesis to strategy for building optoelectronic and energy devices. Renew Sus Ener Rev 135:110391
3. Xu Q, Gao J, Wang S et al (2021) Quantum dots in cell imaging and their safety issues. J Mater Chem B 9:5765–5779
4. Wei Y, Chen L, Zhao S et al (2021) Green-emissive carbon quantum dots with high fluorescence quantum yield: Preparation and cell imaging. Front Mater Sci 15:253–265
5. Pajewska-Szmyt M, Buszewski B, Gadzala-Kopciuch R (2020) Sulphur and nitrogen doped carbon dots synthesis by microwave assisted method as quantitative analytical nano-tool for mercury ion sensing. Mater Chem Phys 242:122484
6. Raji K, Ramanan V, Ramamurthy P (2019) Facile and green synthesis of highly fluorescent nitrogen-doped carbon dots from jackfruit seeds and its applications towards the fluorimetric detection of Au3+ ions in aqueous medium and in vitro multicolor cell imaging. New J Chem 43:11710–11719
7. Xu XY, Ruy R, Ga YL et al (2004) Electrophotographic analysis and purification of fluorescent single-walled carbon nanotube fragments. J Am Chem Soc 126:40
8. Cheng C, Xing M, Wu Q (2019) Green synthesis of fluorescent carbon dots/hydrogel nanocomposite with stable Fe3+ sensing capability. J Alloy Compd 790:221–227
9. Yadav PK, Singh VK, Chandra S et al (2019) Green synthesis of fluorescent carbon quantum dots from azadirachta indica leaves and their peroxidase-mimetic activity for the detection of H2O2 and ascorbic acid in common fresh fruits. ACS Biomater Sci Eng 5:623–632
10. Cheng C, Xing M, Wu Q (2019) Preparation of carbon dots with long-wavelength and photoluminescence-tunable emission to achieve multicolor imaging in cells. Opt Mater 88:352–358
11. Cheng C, Xing M, Wu Q (2019) A universal facile synthesis of nitrogen- and sulfur co doped carbon dots from cellulose-based biowaste for fluorescent detection of Fe3+ ions and intracellular bio imaging. Mater Sci Eng C 99:611–619
12. Chernyak S, Podgornova A, Dorofoev S et al (2020) Synthesis and modification of pristine and nitrogen-doped carbon dots by combining template pyrolysis and oxidation. Appl Surf Sci 507:145027
13. Xu J, Dai L, Zhang C et al (2020) Ionic liquid-aided hydrothermal treatment of lignocellulose for the synergistic outputs of carbon dots and enhanced enzymatic hydrolysis. Bioresour Technol 305:123043
14. Atchudan R, Edison TNJI, Aseer K et al (2018) Highly fluorescent nitrogen-doped carbon dots derived from Phyllanthus acidus utilized as a fluorescent probe for label-free selective detection of Fe3+ ions, live cell imaging and fluorescent ink. Biosens Bioelectron 99:303–311
15. Cui F, Ye Y, Ping J, Sun X (2020) Carbon dots: Current advances in pathogenic bacteria monitoring and prospect applications. Biosens Bioelectron 156:112085

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16. Abbas A, Mariana LT, Phan AN (2018) Biomass-waste derived graphene quantum dots and their applications. Carbon N Y 140:77–99
17. Mahat N, Shamsudin S (2020) Transformation of oil palm biomass to optical carbon quantum dots by carbonisation-activation and low temperature hydrothermal processes. Diam Relat Mater 102:107660
18. Liu G, Li B, Liu Y et al (2019) Rapid and high yield synthesis of carbon dots with chelating ability derived from acrylamide/chitosan for selective detection of ferrous ions. Appl Surf Sci 487:1167–1175
19. Li J, Tang K, Ke J et al (2019) Nitrogen and chlorine co-doped carbon dots as probe for sensing and imaging in biological samples. R Soc Open Sci 6:181557
20. Xue S, Yang Y, Sun Y et al (2019) Photoluminescent lignin hybridized carbon quantum dots composites for bioimaging applications. Int J Biol Macromol 122:954–961
21. Atabaev TS (2018) Doped carbon dots for sensing and bioimaging applications: A minireview. Nanomater 8:342
22. Raji A, Thomas E, Suguna P, Rajangam V (2019) Betel-derived nitrogen-doped multicolor carbon dots for environmental and biological applications. J Mol Liq 296:111817
23. Mehta V, Jha S, Basu H et al (2015) One-step hydrothermal approach to fabricate carbon dots from apple juice for imaging of mycobacterium and fungal cells. Sensor Actuator B Chem 213:434–443
24. Atchudan R, Edison T, Perumal S et al (2020) Eco-friendly synthesis of tunable fluorescent carbon nanodots from Malus floribunda for sensors and multicolor bioimaging. J Photochem Photobiol Chem 390:112–336
25. Xinyue Z, Mingyue J, Na N et al (2018) Natural-product-derived carbon dots: From natural products to functional materials. Chem Sus Chem 11:11–24
26. Sen L, Jingqi T, Lei W et al (2012) Hydrothermal treatment of grass: a lowcost, green route to nitrogen-doped, carbon-rich, photoluminescent polymer nanodots as an effective fluorescent sensing platform for label-free detection of Cu (II) ions. Adv Mater 24:2037–2041
27. Reza M, Samaneh B, Siavash I, Rajjender V (2016) Plant-derived nanostructures: types and applications. Green Chem 18:20–52
28. Swagatika S, Birendra B, Tapas M, Sasmita M (2012) Simple onestep synthesis of highly luminescent carbon dots from orange juice: application as excellent bio-imaging agents. Chem Commun 48:8835–8837
29. Jumeng W, Bitao L, Peng Y (2014) Dual functional carbonaceous nanodots exist in a cup of tea. RSC Adv 4:63414–63419
30. Jie S, Shaoming S, Xiuying C et al (2017) Facile synthesis of fluorescence carbon dots from sweet potato for Fe3+ sensing and cell imaging. Mater Sci Eng, C 76:856–864
31. Jing W, Cai-Feng W, Su C (2012) Amphiphilic egg-derived carbon dots: Rapid plasma fabrication, pyrolysis process, and multicolor printing patterns. Angew Chem 51:9297–9301
32. Bangda Y, Jianhui D, Xue P et al (2013) Green synthesis of carbon dots with down-and up-conversion fluorescent properties for sensitive detection of hypochlorite with a dual-readout assay. Analyst 138:6551–6557
33. Sachdev A, Gopinath P (2015) Green synthesis of multifunctional carbon dots from coriander leaves and their potential application as antioxidants, sensors and bioimaging agents. Analyst 140:4260–4269. https://doi.org/10.1039/C5AN00454C
34. Dan G, Shanoming S, Qin Y, Shen J (2016) Green synthesis of nitrogen-doped carbon dots from lotus root for Hg (II) ions detection and cell imaging. Appl Surf Sci 390:38–42
35. Chatterjee A, Pakrashi S (2003) The treatise on Indian medicinal plants. V-IV. New Delhi. V-IV. New Delhi Natl Inst Sci Commun Inf Resour 128–129
36. Alhede M, Stavnsbjerg C, Bjarnsholt T (2018) The use of fluorescent staining techniques for microscopic investigation of polymorphonuclear leukocytes and bacteria. J Pathol Microbiol Immunol 126:779–794
37. Ao H, Pan HF, Bao Z et al (2018) Synthesis and functionalization of stable and bright copper nanoclusters by in situ generation of silica shells for bioimaging and biosensing. ACS Appl Nano Mater 1:5673–5681
38. Hutter E, Maysinger D (2010) Gold nanoparticles and quantum dots for bioimaging. Nano-Bio-Imaging Anal 74:592–604
39. Thangudu S, Kalluru P, Vankayala R (2020) Preparation, cytotoxicity, and in vitro bioimaging of water soluble and highly fluorescent palladium nanoclusters. Bioengineering 7:20
40. YING L, WARREN D, OLLI H (2004) Tuovinen Fluorescence microscopy for visualization of soil microorganisms—a review. BiolFertil Soils 39:301–311
41. Romero M, Alves F, Stringasci MD et al (2021) One-pot microwave-assisted synthesis of carbon dots and in vivo and in vitro antimicrobial photodynamic applications. Front Microbiol 12:662149
42. Peter A, Julio P, James K (2018) Atkins’ physical chemistry. Oxford University Press
43. Yadav PK, Singh VK, Chandra S et al (2019) Green synthesis of fluorescent carbon quantum dots from Azadirachta indica leaves and their peroxidase-mimetic activity for the detection of H2O2 and ascrobic acid in common fresh fruits. ACS Biomater Sci Eng 5:623–632. https://doi.org/10.1021/acsbiomaterials.8b01528
44. Pal T, Mohiyuddin S, Packirisamy G (2018) Facile and green synthesis of multicolor fluorescence carbon dots from curcumin. In vitro and in vivo bioimaging and other applications. ACS Omega 3:831–843. https://doi.org/10.1021/acsomega.7b01323
45. Arul V, Sethuraman MG (2019) Hydrothermally green synthesized nitrogen-doped carbon dots from Phyllanthus emblica and their catalytic ability in the detoxification of textile effluents. ACS Omega 4:3449–3457. https://doi.org/10.1021/acsomega.8b03674
46. Zhang M, Zhao Y, Cheng J et al (2017) Novel carbon dots derived from Schizonepeta Herba Carbonisata and investigation of their haemostatic efficacy. Artif Cells Nanomedicine Biotechnol. https://doi.org/10.1080/21691401.2017.1379015
47. Wang N, Wang Y, Guo T et al (2016) Green preparation of carbon dots with papaya as carbon source for effective sensing of Iron (III) and Escherichia coli. Biosens Bioelectron 85:68–75. https://doi.org/10.1016/j.bios.2016.04.089
48. Li C-L, Ou C-M, Huang C-C et al (2014) Carbon dots prepared from ginger exhibiting efficient inhibition of human hepatocellular carcinoma cells. J Mater Chem B 2:4564. https://doi.org/10.1039/c4tb00216d
49. Dai J (2019) Nitrogen-doped carbon quantum dots with pinellin ternata as carbon source for high sensitive detection of chromium (vi). Appl Ecol Environ Res 17. https://doi.org/10.15666/aeerr/1705_1213912153
50. Chandra S, Singh VK, Yadav PK et al (2019) Mustard seeds derived fluorescent carbon quantum dots and their peroxidase-like activity for colorimetric detection of H2O2 and ascrobic acid in a real sample. Anal Chim Acta 1054:145–156. https://doi.org/10.1016/j.aca.2018.12.024
51. Zhao X, Liao S, Wang L et al (2019) Facile green and one-pot synthesis of purple perilla derived carbon quantum dot as a fluorescent sensor for silver ion. Talanta 201:1–8. https://doi.org/10.1016/j.talanta.2019.03.095
52. Zhang M, Cheng J, Zhang Y et al (2020) Green synthesis of Zingiberis rhizoma -based carbon dots attenuates chemical and thermal stimulus pain in mice. Nanomedicine 15:851–869. https://doi.org/10.2217/nmm-2019-0369
53. Zhenxiang L, Wanjun W, Liang P et al (2016) Isolation, identification and characterization of novel Bacillus subtilis. J Vet Med Sci 80:427–433
54. Deng H, Yin-Huan LX, Ke-Lin L et al (2017) Chitosan-stabilized platinum nanoparticles as effective oxidase mimics for colorimetric detection of acid phosphatase. Nanoscale 9(29):10292–10300
55. Cullity D (2001) Elements of X-ray Diffraction. Pearson
56. Weiping W, Ya-Chun L, Hong H et al (2014) Facile synthesis of water-soluble and biocompatible fluorescent nitrogen-doped carbon dots for cell imaging. Analyst 139:1692–1696
57. Pin-Che H, Zih-Yu S, Chia-Hsin L, Huan-Tsung C (2012) Synthesis and analytical applications of photoluminescent carbon nanodots. Green Chem 14:917–920
58. Hanjun S, Andong Z, Nan G et al (2015) Deciphering a nanocarbon-based artificial peroxidase: Chemical identification of the catalytically active and substrate-binding sites on graphene quantum dots. Angew Chem 54:7176–7180
59. Mária K, Zoran M, Petr H et al (2018) Carbon quantum dots modified polyurethane nanocomposite as effective photocatalytic and antibacterial agents. ACS Biomater Sci Eng 4:3983–3993
60. Hoffman RA, Forsén S, Gestblom B (1971) Analysis of NMR spectra. Springer, Berlin Heidelberg, Berlin, Heidelberg
61. Jia X, Li J, Wang E (2012) One-pot green synthesis of optically pH-sensitive carbon dots with upconversion luminescence. Nanoscale 4:5572–5575
62. Siavash I, Rajender V (2020) Green synthesis, biomedical and biotechnological applications of carbon and graphene quantum dots. A review. Environ Chem Lett 18:1–25
63. Bányai L, Koch SW (1993) Semiconductor quantum dots. World Scientific
64. Fuller ME, Streger SH, Rothmel RK et al (2000) Development of a vital fluorescent staining method for monitoring bacterial transport in subsurface environments. Appl Environ Microbiol 66:4486–4496

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