Research Article

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Comparative Evaluation of Chemically and Green-synthesized Carbon Quantum Dots for Aquatic Applications

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ABSTRACT

Carbon Quantum Dots (CQDs) are fluorescent carbon-based nanoparticles with a dimension smaller than 10 nanometers. They exhibit enhanced characteristics, including good compatibility, solubility, negligible toxicity, robust inert behavior, high resistance to photo-bleaching, facile modification, electrochemical luminescence, and have potential applications in catalysts, biosensors, imaging, drug delivery, and bio-molecules. Carbon quantum dots were chemically and biologically synthesised from citric acid and unripe lemon as precursors, respectively, through hydrothermal treatment. They were characterized using UV-Vis spectroscopy, UV-Vis transilluminator, SEM, EDAX, and FT-IR. Four Gram-negative fish pathogens, *Aeromonas salmonicida*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*, and four Gram-positive fish pathogens, *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus cereus*, and *Enterococcus faecalis*, were used for the present study. The antibacterial activity was studied by the agar well variant method, and the antioxidant potential using the DPPH assay. Chemically synthesized CQDs showed a larger zone of inhibition against *Pseudomonas fluorescens*, indicating their strong antibacterial activity. The antioxidant activity of CQDs is less than that of standard ascorbic acid.

Keywords: Carbon quantum dots; Antibacterial activity; Antioxidant activity; Gram-negative fish pathogens; Gram-positive fish pathogens.

1. INTRODUCTION

Nanotechnology deals with the handling and transforming characteristics of compounds on the molecular or atomic scale to create innovative gadgets and materials (Pisano and Durlo, 2023). Nanotechnology has applications in the bio-processing industry, computer industry, agro-industry, food industry, poultry and meat industry, fruit and vegetable industry, wine-making industry, textile industry, etc. The key factors driving significant changes in nanoparticle properties are the increased surface area-to-size ratio and the dominance of quantum dot effects at smaller sizes. The larger surface area enhances interactions within nanocomposites, leading to unique properties like improved strength and greater chemical or heat resistance, expanding its use in catalysis and energy storage (Holister *et al.* 2003). Among the remarkable advancements is a new and budding area that merges biotechnology and nanotechnology, known as nanobiotechnology. Nanobiotechnology is rapidly advancing, with quantum dots (QDs) leading its developments in biology and medicine because changing their diameter significantly alters their emission spectra, and also implies that QDs offer tunable, narrow, and symmetric emission spectra with resistance to photobleaching and high temporal stability. When functionalized, QDs can link to biomolecules for selective imaging of subcellular structures (Gao and Xu, 2009). Quantum dots represent an innovative category of nanomaterials that enable researchers to sequence, treat, enhance diagnostics, disease control, and screening processes in the medical field (Malik *et al.* 2023). Quantum dots are nanoparticles with a size range of less than 10 nm.

Carbon quantum dots (CQDs) represent a novel collection of carbonaceous luminescent nanoparticles with a size of precisely smaller than 10 nm. They were unexpectedly found while single-walled carbon nanotubes were purified (Xu *et al.* 2004). They are characterized by low hazard, eco-friendliness, cost-effectiveness, and ease of synthesis. Through surface passivation and functionalization, their properties can be finely tuned, making them highly versatile for use in various applications such as chemical sensing, nanomedicine, bioimaging, electrocatalysis, photocatalysis, and biosensing (Lim *et al.* 2015). The functional group on the surface of the CQDs improves targeting ability, optical properties, and bio-compatibility (Zuo *et al.* 2016). Recently, CQDs have made much progress in preparation, characteristics, and applications (Baker and Baker, 2010). The production of CQDs from

plant materials can convert low-quality substances into active materials that exhibit greater biocompatibility and incorporate different heteroatoms such as nitrogen and sulphur, making them suitable precursors for CQD synthesis (Nazibudin *et al.* 2023). The ascorbic acid and citric acid from lime are one of the carbon-rich sources that are relevant for CQD preparation, and the green-synthesized CQD products possess high stability (Hidayat *et al.* 2024). *Citrus aurantifolia* is rich in citric and ascorbic acid, making it a potent natural carbon source for green synthesis of CQDs (Hidayat *et al.* 2024). The citric acid in citrus fruits undergoes hydrothermal carbonization, resulting in CQDs with well-defined surface functional groups (Wang *et al*. 2017). The green synthesis approach using lemon as a carbon precursor offers a sustainable, non-toxic, and environmentally benign synthesis method, in line with the findings of (Safranko *et al*. 2021).

Antibiotics have remained essential over the past few decades, supporting treatments like orthopaedic and dental implants, organ transplants, and cancer chemotherapy (Shi *et al.* 2020). The mildest form of antibacterial activity involves inhibiting bacterial growth, slowing multiplication to give the host's immune system time to respond. In contrast, the strongest activity results in bacterial death, where the bacteria permanently lose their ability to metabolize and reproduce (Wu *et al.* 2021). The bacterial infection is one of the major causes of fish mortality in aquaculture. The most prevalent Gram-positive bacteria are *Streptococcus agalactiae*, *Lactococcus garvieae*, *Enterococcus faecalis*, and Gram-negative ones are *Aeromonas hydrophila* and *Yersinia ruckeri*, which cause diseases. *Aeromonas hydrophila* is the common pathogen seen in freshwater fishes that is responsible for various pathological conditions. Hence, treatment of bacterial disease using plant-derived phyto-medicals provides greater accuracy than chemotherapeutic agents, as the bacteria are developing resistance against several commercially standard antibiotics. The need for new bioactive substances with antibacterial activity against pathogenic bacteria is trivial (Madhuri *et al.* 2012). Lemon-derived CQDs exhibit potent antioxidant activity due to the polyphenols and flavonoids present in the fruit. These compounds play a vital role in scavenging free radicals, making lemon extract a promising precursor for CQD synthesis (Sharma *et al.* 2020). The antibacterial mechanisms of CQDs highlight generation of reactive oxygen species (ROS) and bacterial membrane disintegration as primary pathways (Akhter *et al.* 2022).

In recent years, CQDs have attracted considerable interest as a multifunctional carbon nanomaterial, presenting exciting possibilities for applications in diverse areas such as electrocatalysis, drug delivery, photodynamic treatment, photocatalysis, bioimaging, and chemical sensing (Yan *et al.* 2019). The carbon quantum dots show excellent antibacterial and antioxidant activity (Wen *et al.* 2023). Antibacterial activity can be studied by agar diffusion and agar well variant methods (Valgas *et al.* 2007). Antioxidants neutralize free radicals, which harm biological cells and negatively affect living organisms. Antioxidants are classified as primary or secondary based on their mechanism, inhibiting oxidation by donating or accepting hydrogen to form a stabilizer radical (Munteanu and Apetrei, 2021). A paper-based DPPH assay was developed as a cost-effective method requiring minimal reagents and sample volumes, suitable for rapid-processing analysis of antioxidants. The CQDs have shown strong antibacterial effects against Gram-positive, Gram-negative, and drug-resistant bacterial strains (Hao *et al.* 2023). They possess strong antioxidant activity, efficiently scavenging free radicals due to a specified structure and functional group on the surface, with negligible cytotoxic effects and excellent physiological tolerance. This makes it vital for applications in healthcare and food science, with its activity measurable *via* assays like DPPH radical scavenging tests.

The potential of carbon quantum dots synthesized using lime extract, using a one-pot hydrothermal method, is explored, emphasizing their eco-friendly approach and characteristic properties. The comparison analysis with that of chemically synthesized CQDs on antioxidant and antibacterial activity against fish pathogens is presently significant. Consequently, this work focused on analyzing the properties and applications of the material.

2. MATERIALS AND METHODS

All the laboratory-grade chemicals were collected from the Department of Biology, The Gandhigram Rural Institute (Deemed to be University), Gandhigram, Tamil Nadu, India. The bacterial strains were collected from the National College, Tiruchirappalli, Tamil Nadu, India. Fresh and green unripe lemons were collected directly from a farm in Sengettampatty Village, Dindigul, Tamil Nadu, India.

2.1 Synthesis of Chemical CQDs

The carbon quantum dots were chemically prepared through the hydrothermal method by mixing 2 g of citric acid and 0.7 g of L-histidine in 50 mL of distilled water. The solution was kept in a magnetic stirrer for 10 minutes to get a uniform dissolved solution, placed in a Teflon autoclave, and dried at 180°C for 8 hours. The brown color indicates the presence of CQDs in the solution. The solution was then centrifuged at 10000 rpm for 20 minutes at 4°C. The aliquots were collected in a syringe and filtered using a 0.2-micron filter paper (Nammahachak *et al.* 2022). The filtrate was used for the characterization of CQDs, and the remaining ones were wrapped using aluminum foil to maintain the fluorescence and preserved at 4°C for further use (Fig. 1).

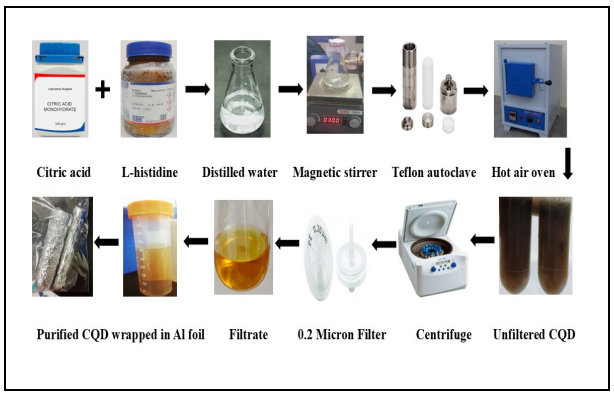


Fig. 1: Chemical synthesis of CQDs

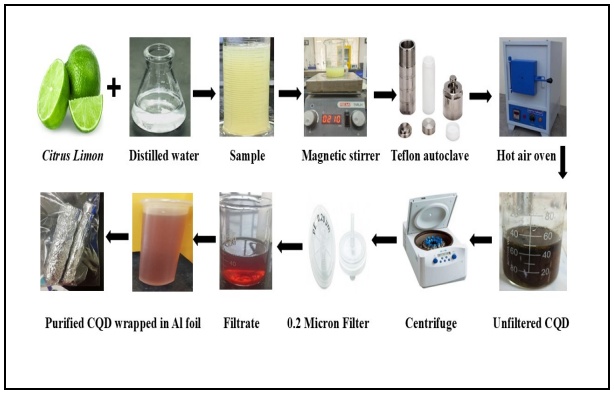


Fig. 2: Green synthesis of CQDs

2.2 Preparation of Lemon Extract

The unripe lemons were rinsed twice with distilled water to remove the dust and dirt. A sieve was used to get rid of the seeds and pulp. The pulp-free lemon juice was extracted and added with distilled water in the ratio of 1:2. The sample was then stirred at 200 rpm for 15 minutes to ensure thorough mixing and homogenization. The lemon juice was collected in a clean glass container and used for the synthesis of CQDs.

2.3 Synthesis of Green CQDs

The carbon quantum dots were synthesized through the hydrothermal method in an autoclave, as shown in the Fig. 2. The lemon juice was extracted and then poured into a Teflon-coated autoclave, which is sealed and dried at 180°C for 8 hours to facilitate carbonization. This process led to the formation of a dark brown solution, which is an indication of CQD formation. Once the autoclave was cooled to room temperature, the dark brown solution obtained was centrifuged at 10,000 rpm for 10 minutes to remove larger carbon residues. This centrifugation step was repeated twice to ensure the elimination of non-carbonized particles. The supernatant containing CQDs was collected and further filtered with a 0.22-µm filter paper to remove unreacted compounds and large aggregates. The purified CQD solution was wrapped in an aluminum foil and stored at 4°C for further characterization and bioactivity studies. This is an eco-friendly and controllable approach to produce CQDs with high quantum yield without requiring additional post-synthesis modifications.

2.4 Characterization

The chemical and green synthesized CQDs have were confirmed of their presence using various characterization techniques. The absorption spectrum and luminous property of samples were detected using a UV-Visible spectrophotometer and a UV-Visible transilluminator. The morphology and elemental features were carefully studied using Scanning Electron Microscopy (SEM), Energy-Dispersive X-ray analysis (EDX), and Fourier Transmission Infrared Spectroscopy (FT-IR).

2.5 Antibacterial Activity

For the present study, four Gram-negative fish pathogens, *Aeromonas salmonicida*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*, and four Gram-positive fish pathogens, *Bacillus cereus*, *Enterococcus faecalis*, *Micrococcus luteus*, and *Staphylococcus aureus*, were selected (Table 1). The pathogens in stock were subcultured to get a pure culture of pathogens. In the agar well variant method, the inoculum of pathogens was uniformly spread on MH agar in a sterile Petri dish using a cotton swab. Five wells (7 mm in diameter and 20 mm apart) were created in each Petri dish. Three wells were filled with different concentrations of the sample solution, one with a positive control and another with a negative control. Cultures were incubated at 36°C for one day under aerobic conditions. The zone of inhibition was calculated in mm after observing confluent pathogenic growth. The tests were performed in triplicate.

Table 1. Culture code of fish pathogens

Fish Pathogens	Culture Code
Aeromonas salmonicida	NCT 338
Pseudomonas fluorescence	NCT 48
Pseudomonas aeruginosa	NCT 114
Stenotrophomonas maltophilia	NCT 259
Staphylococcus aureus	NCT 45
Micrococcus luteus	NCT 24
Bacillus cereus	NCT 11
Enterococcus faecalis	NCT 34

2.6 Antioxidant Activity

The antioxidant activity of CQDs was studied through DPPH assay. The DPPH is colored, but when it reacts with electrons of an antioxidant compound, it becomes colorless. The sample solution was mixed with methanol in different concentrations in the test tubes. Ascorbic acid was used as the standard, methanol was used as blank, and DPPH with methanol was used as control. The changes in the color after adding DPPH were measured by UV-Vis spectroscopy at 517 nm. Antioxidant activity was measured by the following equation (1):

$$\text{Antioxidant activity} = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100 \ldots (1)$$

3. RESULTS AND DISCUSSION

3.1 UV-vis Spectroscopy

The spectrum of chemically synthesized CQDs is shown in Fig. 3. The UV–Visible absorption spectrum of the synthesized CQDs exhibited two characteristic peaks at 290 nm and 350 nm. The absorption peaks at 290 nm correspond to the π⟶π* transition of C=C bonds, while the peak at 350 nm is attributed to the n⟶π* transition of C=O bonds. These spectral features indicate the presence of conjugated carbon structures and oxygen-containing functional groups on the surface of the CQDs. Similar observations were reported by Yadav *et al.* (2023) and Bandi *et al.* (2018) confirming the aromatic and oxygen-rich nature.

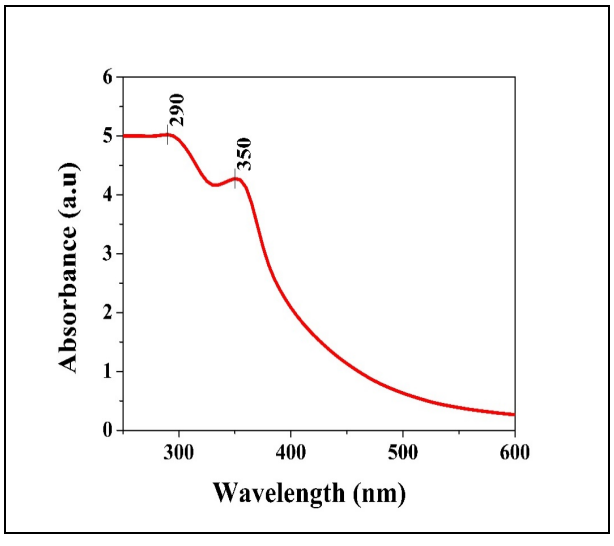


Fig. 3: UV-visible absorption spectrum of chemically synthesized CQDs

Fig. 4 depicts the UV absorption spectrum of green synthesized CQDs. The peak around 295 nm is associated with π⟶π* transitions of C=C bonds in aromatic sp^2 domains within the carbon core, while the peak around 355 nm arises from n⟶π* transitions of C=O groups and other oxygen-containing surface functionalities. The absorption features confirm the presence of a conjugated carbon structure and surface passivation, characteristics of well-formed CQDs with photoluminescent properties. Jadhao *et al.* (2008) found that CQD synthesized by hydrothermal method exhibited absorption edge values ranging from 250 to 350 nm. Dey *et al.* (2014) also noticed a prominent peak at a wavelength of 280 nm, linked to the π⟶π* orbital transition resulting from the sp^2 hybridization of carbon–carbon double bonds (C=C).

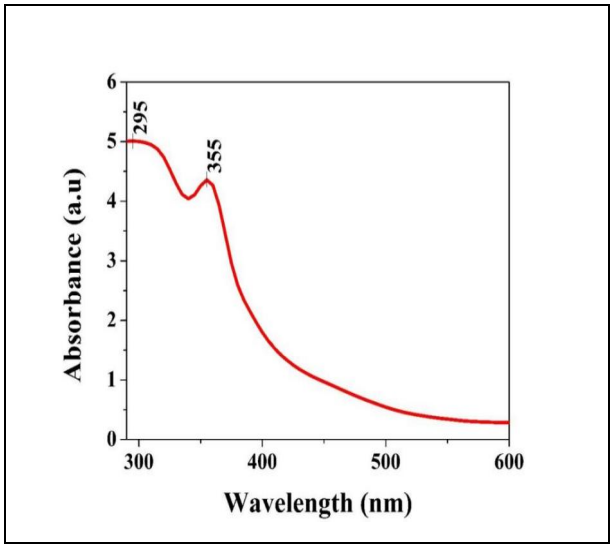


Fig. 4: UV-visible absorption spectrum of green synthesized CQDs

3.2 UV-vis Transilluminator

Under UV light from a UV-Visible transilluminator, the synthesized CQDs displayed a prominent green fluorescence (Fig. 5). This visible emission confirms the successful formation of photoluminescent CQDs with effective surface passivation and quantum confinement effects. The green fluorescence indicates the specific surface states or functional groups that influence the emission wavelength, highlighting the potential in fluorescent labeling, sensing, and imaging.

Jelinek (2016) detected the fluorescence intensity in carbon quantum dots produced using citric acid and coated with polyethylene glycol as the carbon precursor for the study of DNA. Also, fluorescence was detected as green luminescence for CQDs synthesized by chemical synthesis (Milosavljevic *et al.* 2015).

The CQDs synthesized from lemon juice exhibited green fluorescence under UV-VIS transillumination (Fig. 6). This fluorescence is attributed to surface functional groups like hydroxyl, carboxyl, and

amino groups, which enhance light emission by preventing non-radiative recombination.

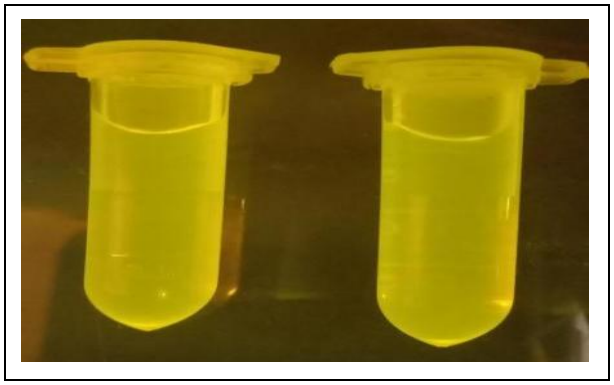


Fig. 5: UV-vis transilluminated image of chemically synthesized CQDs

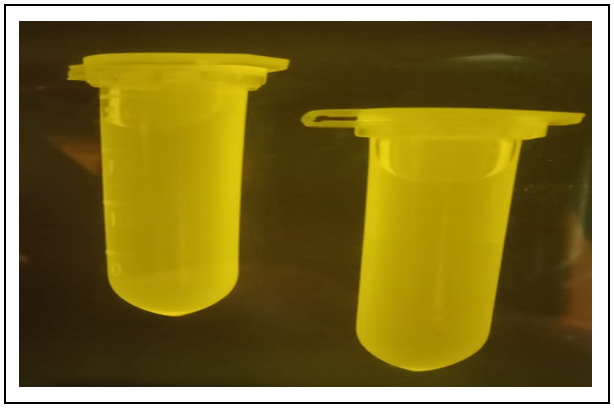


Fig. 6: UV-vis transilluminated image of green synthesized CQDs

3.3 SEM Analysis

The SEM analysis of the chemical and green synthesized CQDs at a 2 µm scale are depicted in Fig. 7 and Fig. 8, which show a uniform surface morphology with well-dispersed, spherical particles. While individual CQDs were not clearly resolved due to their nanoscale size, the smooth and non-aggregated texture suggests successful synthesis and good stability. The SEM images reveal curved sheets with size of hundred micrometers, similar to the work of Malavika *et al.* (2024). The small size of the particles caused them to aggregate, leading to the formation of larger chunk-like structures. The spherical shape indicates effective particle formation through the hydrothermal method and also suggests that the CQDs synthesized from lemon have well-defined edges, which may contribute to their stability in aqueous solutions and minimize aggregation. The high surface energy resulting from the very small size of the carbon particles contributes to their aggregation, resulting in a morphology of particles measuring less than 10 nm, as also noticed by Sabet and Mahdavi (2019). The morphological analysis by SEM revealed that the CQDs exhibited a predominantly spherical shape, which is consistent with prior reports (Yadav *et al.* 2023; Li *et al.* 2020).

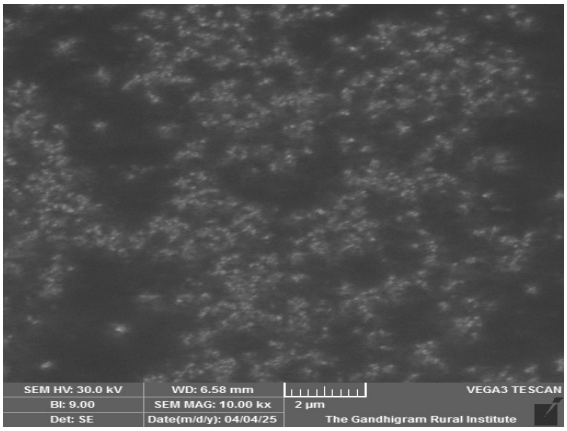


Fig. 7: SEM image of chemically synthesized CQDs

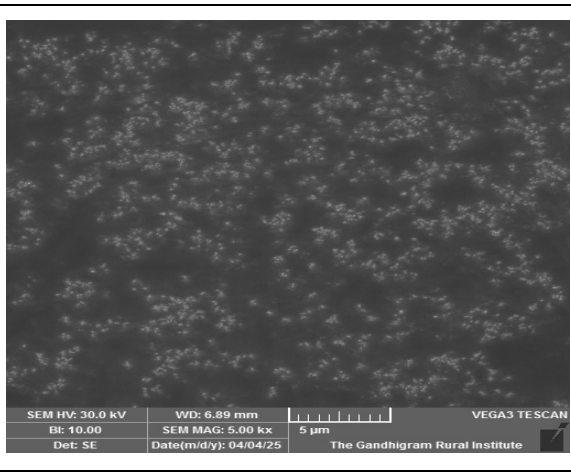


Fig. 8: SEM image of green synthesized CQDs

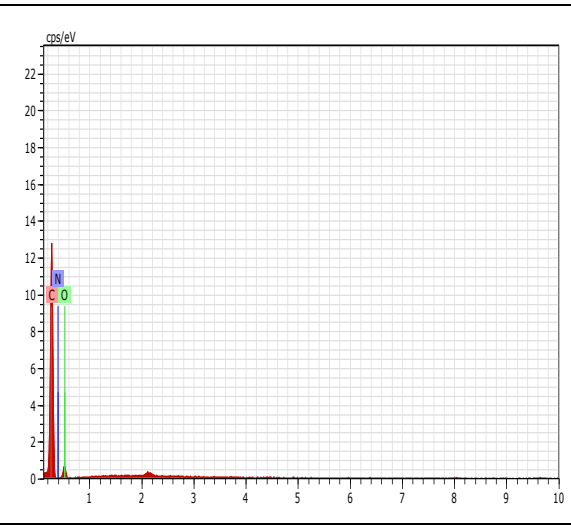


Fig. 9: EDX patterns of chemically synthesized CQDs

3.4 EDX Analysis

The elemental analysis shows that the chemically synthesized CQDs consist of C (78.30 wt%, 82.27 at%), O (16.20 wt%, 12.78 at%), and N (5.50 wt%, 4.95 at%) (Fig. 9 and Table 2). The high carbon content suggests a well-structured carbon core, which is crucial for quantum effects. The O and N indicate the incorporation of functional groups such as hydroxyl, carboxyl, and amine groups on the CQDs. These functionalities improve water solubility, enhance photoluminescence, and provide chemical stability.

Table 2. EDX analysis of chemically synthesized CQDs

Elements	Weight Percentage (wt.%)	Atomic Percentage (%)
Carbon	78.30	82.27
Oxygen	16.20	12.78
Nitrogen	5.50	4.95

Elemental composition analysis by EDAX confirmed the presence of carbon (78.46%), oxygen (15.69%), and nitrogen (5.86%) in the green-synthesized CQDs (Fig. 10 and Table 3). The high carbon content confirms the carbonaceous nature of the CQDs, while oxygen suggests the presence of oxygen-containing functional groups such as hydroxyl, carbonyl, or carboxyl groups. The presence of nitrogen in trace amounts may result from naturally occurring amino acids or proteins in the lemon extract that, altogether, enhance the chemical reactivity of the dots. Similar dominant peaks for C and O in green synthesized CQDs, confirming the structure of the carbon dots, were reported earlier (Sharma *et al.* 2020; Safranko *et al.* 2021; Kumar *et al.* 2023).

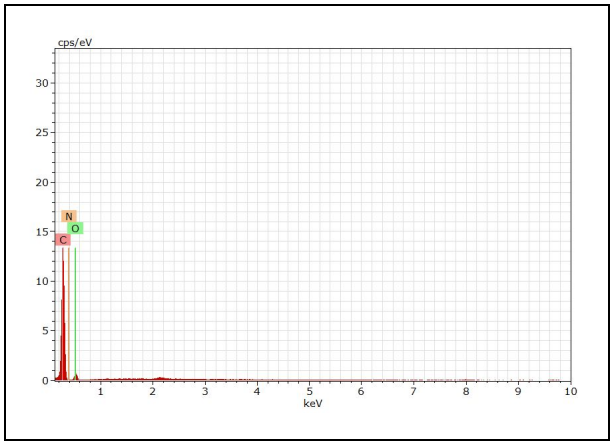


Fig. 10: EDX patterns of green synthesized CQDs

Table 3. EDX analysis of green synthesized CQDs

Elements	Weight Percentage (wt. %)	Atomic Percentage (at. %)
Carbon	78.46	82.37
Oxygen	15.69	12.36
Nitrogen	5.86	5.27

Table 4: FT-IR band representation of functional groups in chemically synthesized CQDs

Bands	Functional Groups	Vibration Mode	Intensity
3330.40	Hydroxyl group	O-H/N-H Strech	Strong or Board
1635.31	Carbonyl group	C=C	Medium to strong
652.71	Aromatic group	C=H	Weak to Medium

3.5 FT-IR Analysis

The FTIR analysis of the chemically synthesized CQDs is depicted in Fig. 11, exhibiting prominent bands of absorption at 3330.46 cm^{-1}, 1635.34 cm^{-1}, and 652.78 cm^{-1}. The peak at 3330.46 cm^{-1} is related to the stretching vibrations of O-H and N-H groups, indicating the presence of hydroxyl or amine functionalities on the surface. The band at 1635.34 cm^{-1} corresponds to C=C or C=O stretching vibrations, suggesting the presence of conjugated carbon structures or carbonyl groups. The absorption at 652.78 cm^{-1} is associated with C-H bending or out-of-plane deformation vibrations, typically found in aromatic or substituted hydrocarbon rings. These functional groups confirm successful surface functionalization, contributing to the solubility and photoluminescence of the CQDs (Table 4). The observations are consistent with reported studies (Chen *et al.* 2016; Mahmoud *et al.* 2020).

The FTIR spectrum of the biosynthesized CQDs showed characteristic peaks at 3341.08 cm^{-1}, 1636.33 cm^{-1}, and 647.01 cm^{-1} (Fig. 12; Table 5), indicative of the presence of various surface functional groups that contribute to the hydrophilicity and biological activity of the CQDs. The broad absorption around 3341 cm^{-1} is attributed to O–H and/or N–H stretching vibrations, indicative of surface hydroxyl and amine groups, consistent with previous reports on plant-derived CQDs (Pant *et al.* 2023; Zhai *et al.* 2023). The peak at 1636 cm^{-1} represents the C=O stretching of carboxyl groups, a typical feature of oxidized carbon nanomaterials (Wang *et al.* 2017), and the band at 647 cm^{-1} is attributed to C–

H bending, reflecting the presence of aromatic rings or unsaturated hydrocarbon structures (Tadesse *et al.* 2020).

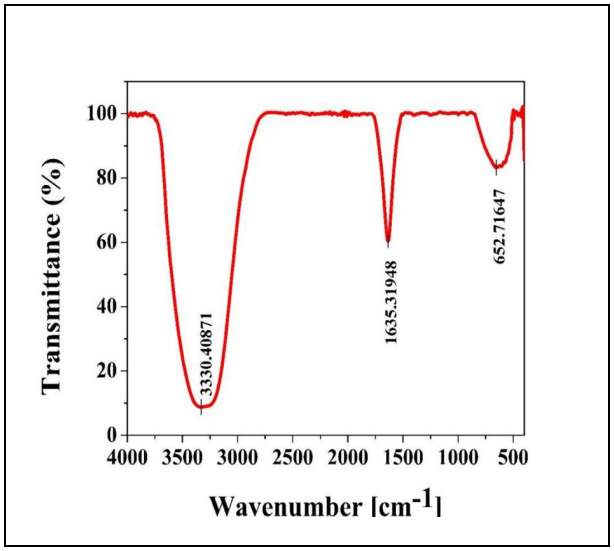


Fig. 11: FT-IR pattern of chemically synthesized CQDs

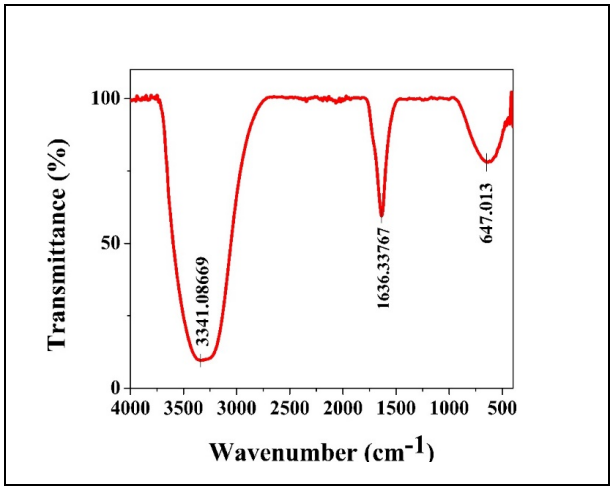


Fig. 12: FT-IR pattern of green synthesized CQDs

Table 5. FT-IR band representation of functional groups in green synthesized CQDs

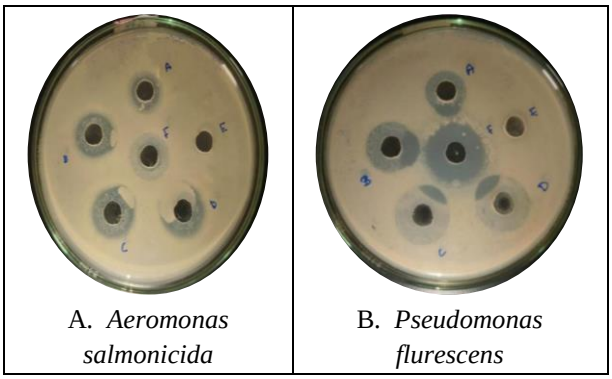
Bands	Functional Groups	Vibration Mode	Intensity
3341.08	Hydroxyl/ Amine	O-H / N-H Stretch	Strong
1636.33	Carbonyl	C=O	Medium
647.01	Alkenes	C-H Bend	medium

3.6 Antibacterial Activity

The antibacterial activity of chemically synthesized CQDs was assessed against eight fish pathogens, including four Gram-negative bacteria such as *Aeromonas salmonicida*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* (Fig. 13) and four Gram-positive bacteria such as *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus cereus*, and *Enterococcus faecalis* (Fig. 14) at concentrations of 25, 50, 75, and 100 µL. The CQDs demonstrated significant antibacterial activity at higher concentrations, with the strongest inhibition observed against Gram-negative pathogens, especially *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, showing inhibition zones similar to the positive control (kanamycin). Gram-positive bacteria exhibited moderate antibacterial activity, which increased with concentration. The negative control (distilled water) showed no antibacterial effects, confirming that the observed activity was due to the CQDs. The results suggest that CQDs possess potent antibacterial properties, particularly against Gram-negative pathogens, making them promising candidates for controlling bacterial infections in aquaculture. The results are consistent with the findings of Li *et al.* (2020).

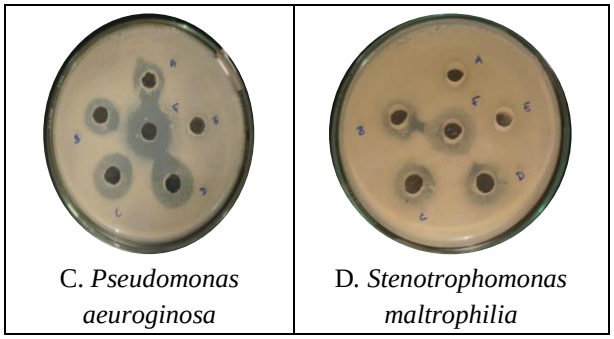
According to Yabalak *et al*. (2025), the green produced CQDs have the ability to demonstrate strong antibacterial activity against both Gram-positive and Gram-negative bacteria. With minimum inhibitory concentration values of 1.2 mg/mL and 1.1 mg/mL, respectively, the nitrogen-doped CQDs made from *Solanum nigrum* showed significant antibacterial effectiveness against the bacterial strains *S. aureus and E. coli* (Shen *et al*. 2025). With the lowest minimum inhibitory concentrations (15.62 µg/mL) against *E. coli and Candida*, the CQDs made from artichoke leaves demonstrated their effectiveness against these pathogens (Diab *et al*. 2025). The reasons behind growth inhibition can be understood as membrane lysis occurring as a result of interaction between the positive charges on the surface of CDs and negative charges on the cell membranes of the organism (Vargheese *et al.* 2021).

Overall, the study highlights the efficacy of carbon quantum dots synthesized from lemon juice exhibited strong fluorescence, significant antioxidant activity, and potent antibacterial effects against fish pathogens. The surface functional groups contributed to its stability and biological activity.



A. *Aeromonas salmonicida*

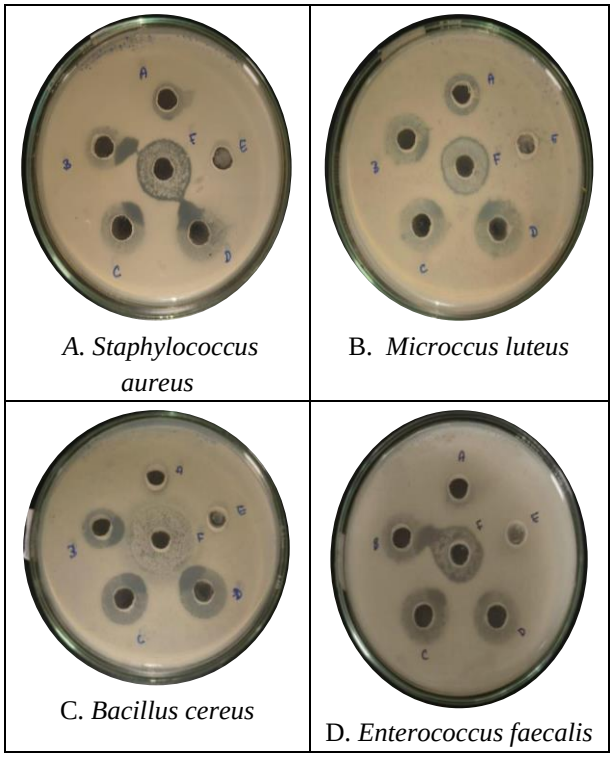
B. *Pseudomonas flurescens*



C. *Pseudomonas aeuroginosa*

D. *Stenotrophomonas maltrophilia*

Fig. 13: Antibacterial activity of chemically synthesized CQDs against gram-negative bacteria



A. Staphylococcus aureus

B. *Micrococcus luteus*

C. *Bacillus cereus*

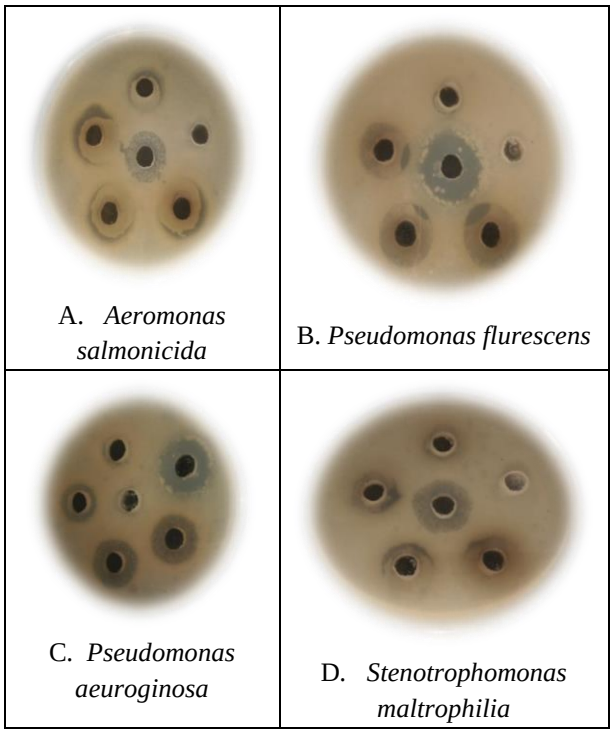
D. *Enterococcus faecalis*

Fig. 14: Antibacterial activity of chemically synthesized CQDs against Gram-positive bacteria

3.7 Antioxidant Activity

The antioxidant activity of the CQDs and that of ascorbic acid, presented in Fig. 17, was evaluated using the DPPH radical scavenging assay, with absorbance recorded at 517 nm. The reduction in absorbance intensity at 517 nm correlates with the concentration-dependent quenching of DPPH radicals, confirming the antioxidant potential of the CQDs. The progressive decrease in DPPH absorbance suggests that by donating electrons or hydrogen atoms, CQDs effectively scavenge and neutralize free radicals. A notable antioxidant potential of the CQDs, which can be related to the presence of functional groups such as hydroxyl, carboxyl, and amine groups. In chemical and green synthesized CQDs, the results demonstrated a concentration-dependent increase in radical scavenging activity, respectively. At a lower concentration of 20 µl, the chemical CQDs exhibited a scavenging activity of 10.38% and green CQDs 29.86%, which progressively increased with concentration. The activity reached 27.45% in chemical CQDs and 51.34% in green CQDs at 40 µl. At 60 µl chemical CQDs reached 35.57% and green CQDs to 77.85%. At higher concentrations, the chemical and green CQDs showed maximum scavenging capacities of 47.56% and 79.53% at 80 µg/mL and 58.38% and 80.53% at 100 µl respectively. In comparison to the standard, ascorbic acid exhibited superior scavenging activity across all concentrations tested. At 10 µl, ascorbic acid showed 20.46% activity, which increased steeply to 76.89% at 20 µl and remained consistently high, reaching a maximum of 86.57% at 100 µl.

The high scavenging activity regarding a huge number of electrons donating to the surface functional groups, including carboxyl and hydroxyl groups were observed in the antioxidant activity of CQD derived from coconut husk (Chunduri *et al.* 2016). Different concentrations of carbon quantum dots were taken from 10 to 100µl (Safranko *et al*., 2021; Pant *et al*., 2023). CQDs synthesized from *Azadirachta indica* of varying concentrations (5–50 µg/mL) were examined to determine the total antioxidant capacity and observed a gradual increase from 108.59% to 148% as a result of increasing intensity from pale green to greenish (Gedda *et al*. 2023).



A. *Aeromonas salmonicida*

B. *Pseudomonas flurescens*

C. *Pseudomonas aeuroginosa*

D. *Stenotrophomonas maltrophilia*

Fig. 15: Antibacterial activity of green synthesized CQDs against Gram-negative bacteria

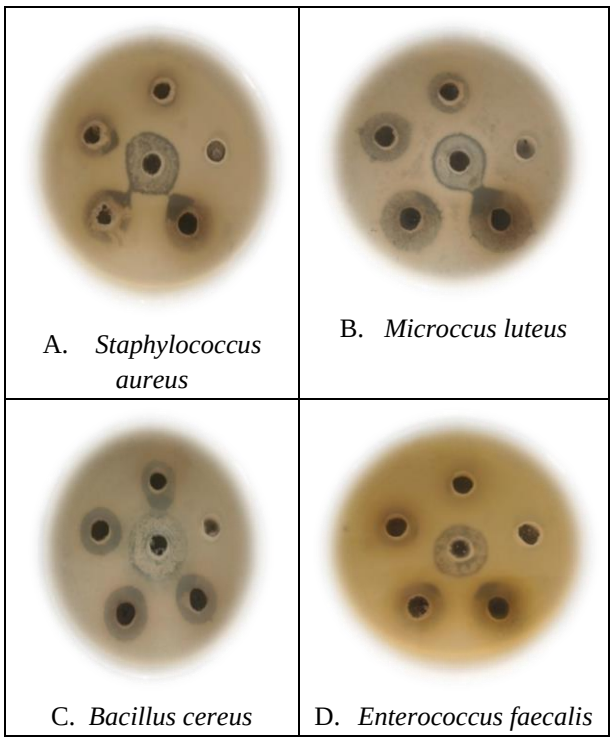


Fig. 16: Antibacterial activity of green synthesized CQDs against Gram-positive bacteria

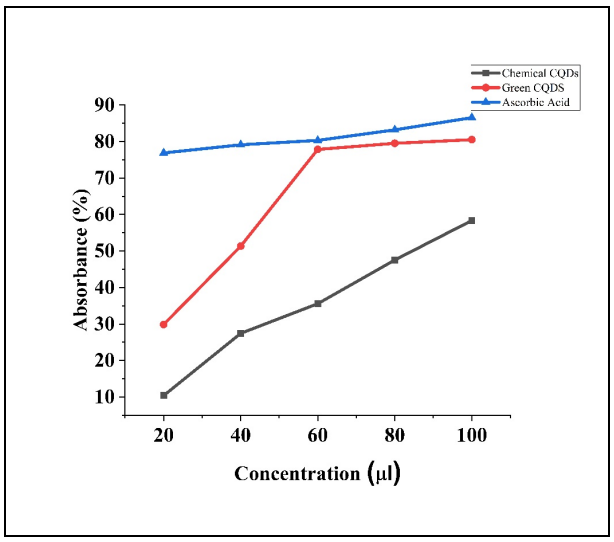


Fig. 17: Antioxidant activity

4. CONCLUSION

The CQDs were synthesized using a facile hydrothermal method, with citric acid employed as the carbon precursor in chemical synthesis and unripe lemon as a source for bio synthesis. The CQDs are shown as broad-spectrum antibacterial and antioxidant agents, demonstrating potent activity against fish pathogens. The results depict that bio-synthesized carbon quantum dots exhibit more microbial activity compared to those synthesized chemically. Due to their simple, cost-effective, and one-step hydrothermal preparation, along with the favorable biocompatibility and reduced cytotoxicity, the CQDs hold promise for advancing bacterial research and clinical applications. This study illustrates the effectiveness of carbon quantum dots in bacterial strains. Optimizing extract concentration and reaction time can improve the effectiveness of CQD synthesis, providing important insights for future biomedical and antimicrobial applications and antioxidant potentials.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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