

CITRIC ACID BIOFUNCTIONALIZED CARBON DOTS AS A POTENTIAL FLUORESCENCE TOOL IN THE DIAGNOSIS OF DISEASES

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ABSTRACT

Carbon dots (CDs) or carbon quantum dots are zero- dimensional nanomaterials that have excellent fluorescent properties. Due to their excellent optical properties as well as their other properties such as biocompatibility, low toxicity, photoluminescent and simple synthesis methods they have emerged as promising nanomaterials in disease diagnosis, photodiode, drug delivery, solar cells and many other applications. In the current study, CDs were synthesized from orange peel which are economical, abundant and renewable biomass which has carbon and other functional groups such as hydroxyl and carboxyl groups making it the ideal precursor for green and sustainable synthesis. To determine their optical and structural properties, the CDs were characterised using XRD, Raman spectroscopy, TEM, UV-Visible spectroscopy and FTIR and it also showed bright blue fluorescence under UV illumination. Also, their interaction with DNA was explored to study their complex formation while their bioimaging potential was demonstrated against harmful bacteria such as *Klebsiella pneumoniae* and *Staphylococcus aureus*. Hence, these sustainably derived CDs can be used in various application such as gene delivery, bioimaging and a diagnostic tool.



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I. INTRODUCTION

Carbon has a unique ability to showcase multiple orbital hybridizations which helps it to form various chemical bonds with diverse orientations making it the basic building block of life. This diversity of carbon makes it to bond with other elements allowing it to create various molecules and compounds with differing physical and chemical properties [1]. Graphene, Carbon dots, Carbon nanotubes and Carbon black are various forms of carbon with varying form of nanostructures and properties. Due to their exemplary mechanical, chemical, thermal and optical characteristics, carbon nanoparticles have found exceptional usage in nanotechnology applications [2]. Carbon nanoparticles with size less than 20nm are referred to as carbon dots. They have a unique property of fluorescence. They have various applications such as bioimaging and biosensing due to their biocompatible and hydrophilic carbon groups [3]. Based on their unique formation mechanisms, nanostructures and properties, they are categorized as graphene quantum dots (GQDs), carbon nanodots (CNDs) and carbonized polymeric dots (CPDs). Absorbing property of carbon dots across the ultraviolet wavelength extending into the visible spectrum is another exceptional property of carbon dots. Longer wavelength UV absorption, often showing as a weaker peak between 300 and 400 nm, is ascribed to $n \rightarrow \pi^*$ transitions of C=O bonds. On the other hand, shorter wavelength UV absorption, spanning approximately between 200 and 350 nm, is attributed to $\pi \rightarrow \pi^*$ electronic transitions of C=C and C=N bonds [4]. The photoluminescence characteristics of carbon dots are among their most alluring features. This feature has enabled carbon dots to be used in various applications like bioimaging and biosensing. The most common method of doping carbon dots is synthesis using precursors that contain N. The kind of heteroatoms can adjust the emission wavelength. Individual N forms were shown to cause both red and blue shifts, which is explained by the effect's dependence on N type and position [5].

I.1 FABRICATION USING HYDROTHERMAL TECHNIQUE

Carbon dots are produced using two methods namely, top-down or bottom-up synthesis. The top-down method is usually employed for large carbon materials like soot and graphene in which these carbon sources will reduce into nano-sized carbon dots when they are heated [6]. Bottom-up approach is a commonly used synthesis method for carbon dots as it is suitable for mass production, flexibility (both in terms of precursors and synthesis routes), cost effective and eco-friendly. Bottom-up approach will lyse small organic carbon sources into smaller nano sizes either by using hydrothermal method or microwave-based approach [7]. The current investigation involved a bottom -up synthesis method called hydrothermal process for synthesizing carbon nanoparticles from orange peel. The chemical reaction will take place by adding the carbon source (orange peel) and the solvent (methanol) in a sealed pressure vessel. Different types of precursors such as milk, aloe vera, papaya etc. were successfully converted into carbon dots using hydrothermal method [8].

I.2 ORANGE PEEL FOR THE FABRICATION OF CARBON DOTS

Orange range in total sugar content from 29% to 44%, with soluble and insoluble carbohydrates being the most prevalent and commercially valuable components of this residue. Apart from the carbohydrates, citric acid is one of the major components of orange peel. Citric acid is a weak organic compound that can be used to synthesize a fluorescence emitting carbon dots. Carbon dots synthesized from citric acid will usually have a blue emission [9]. Solvents might be a significant factor in carbon dot photoluminescence. Carbon dot's surface emissive defect or solvent adherence can both cause tunable luminescence in different solvents [10].

I.3 APPLICATION OF CARBON DOTS

Nanoparticles known as carbon dots exhibit distinctive structures and properties, garnering significant attention from researchers in areas of applications spanning from sensing of biological agents, precise and targeted drug delivery, photodynamic based therapy, degradation of molecules using photocatalysis, and in energy harvesting solar cells in recent years [11]. Because of their exceptional properties like high degree of anti-swelling and anti-photobleaching, as well as non-toxicity and considerable biological compatibility, they find wide applications in biomaterials. They exhibit tunable permeability, weak protein interactions, stealthing action from immune system, safe bodily clearance and their cost-effectiveness renders the carbon dots as a potential candidate for employing as in fluorescence labelling and imaging applications. These nanoparticles have been utilised in Parkinson's disease and Alzheimer's disorder research recently as a multifunctional drug, drug carrier, and imaging agent because of their increased blood brain barrier penetration ability, adjustable surface functionalization, distinct optical property, biocompatibility, enhanced drug encapsulation ability, and ability to release a sufficient amount of drug at targeted protein aggregates. Carbon dots can be used for locating and identifying the various types of tumours and characterize them for further clinical diagnosis.

II. MATERIALS AND METHODOLOGY

II.1 CARBON DOT SYNTHESIS FROM ORANGE JUICE AND ORANGE PEEL

The citric acid from orange juice extract is prepared by squeezing the orange and filtering the juice. Orange extract of 45 ml volume is mixed with 5 ml of methanol. The mixture is poured into a 100ml Teflon set up. The set up is heated under pressure in a furnace for 2 hrs. After cooling at 4°C, the sample was washed thrice at 7000 rpm for 10 min. The resulting supernatant is collected and visualized under UV chamber. Mortar and pestle was used to crush 1g of cleaned orange peel into a paste. The paste is then dissolved in 100 ml of distilled water using magnetic stirrer for 30 minutes. Post stirring, Whatman filter paper was used to obtain the pure filtrate. In a 200ml Teflon setup, 10 ml of methanol and 90ml of the extract are then combined. The setup was kept in a furnace for heating for 2 hours. After cooling the sample was centrifuged for 7000 rpm at 4°C for 10 minutes. Under the UV chamber, the supernatants were collected and visualized.

II.2 OPTIMIZATION OF CARBON DOT ANALYSIS

The optimization technique for carbon dots comprises the modification of temperature, a significant aspect determining their fluorescence qualities. This work focuses on studying the impact of variations in temperature on the fluorescence emission of carbon dots produced from orange extracts. The intensity of fluorescence exhibited by the carbon dots were studied and optimized at three distinct temperatures-200°C, 160°C, and 140°C.

II.3 CHARACTERISATIONS

II.3.1 UV-Visible Spectroscopy Analysis

The optical properties and potential applications of the carbon dot were studied using Shimadzu UV- 1800 instrument to investigate the absorption characteristics of the carbon dots.

II.3.2 Fourier Transform Infrared Spectroscopy (FTIR) Studies

The analysis of the functional groups in the carbon dot solution was studied using FTIR (Fourier Transform Infrared Spectroscopy) which is an instrument manufactured by Bruker Germany. This strong analytical method permits the analysis of molecular vibrational modes, aiding the analysis of specific functional moieties available in the synthesized sample.

II.3.3 Transmission Electron Microscopy (TEM)

The ThermoScientific Talos F200S, G2 high-performance TEM model was used for high-resolution imaging and analysis of materials and is particularly useful for the characterization of synthesized carbon dots.

II.3.4 X-Ray Diffraction (XRD) Analysis

This non-destructive method X-ray diffraction analysis (XRD) is employed to elucidate various aspects of crystalline powders, including its chemical composition, crystallographic structure, and physical properties. Through the interaction of X-rays with the atomic structure of the material, XRD provides valuable insights into its internal arrangement and properties without causing damage to the sample.

II.3.5 Carbon Dots for Bioimaging Microorganisms

Klebsiella pneumoniae and *Streptococcus aureus* were used for the bioimaging application. *S.aureus* and *Klebsiella* were grown in 50 ml nutrient broth. The cultures were placed in the shaking incubator until their OD value reached 0.5 at an absorbance of 600nm. Once the cultures reached an OD of 0.5, 30 ml of the cultures were centrifuged at a temperature of 4°C for 5 minutes at 2000 rpm. The pellets were obtained by discarding the supernatant and washed them thrice using nutrient media and then by distilled water by centrifuging the cells using the same condition. In the *S.aureus* and *Klebsiella* cells, 5 ml of carbon dots were added to the cell and incubated for 1hr. The stained bacterial cells were washed using distilled water and visualized using fluorescence microscopy.

II.3.6 Analyses of DNA-Carbon Dot Interaction Mechanisms

The synthesized carbon dot was mixed with the DNA at equal ratio and vortexed for 1 minute. The solution was then incubated for 30 minutes to obtain a DNA-carbon dot complex. To confirm the formation of the complex, the sample was loaded in a 0.8% agarose gel. As control, another DNA sample along with loading dye was loaded in another well. The gel was run at 100V for 1 hour before visualizing it under UV.

III. RESULTS AND DISCUSSION

III.1 FABRICATION & OPTIMIZATION OF CARBON DOT ORANGE JUICE AND ORANGE PEEL AT 200°C

For the first batch of synthesis, both the orange peel and orange juice were heated at 200°C in a furnace for 2 hours. However, there was no emission under UV for both samples. After heating the orange juice extract at 200°C, significant amounts of black soot were formed and there was a significant amount of volume reduction compared to the peel extract, both of which are signs of excessive carbonization during the heating process (Fig.1). Orange juice's high concentration of simple sugars like glucose and fructose, which easily undergo dehydration and polymerization at high temperatures and result in the formation of bulk carbonaceous residues rather than nanoscale fluorescent carbon dots, may be the cause of this phenomenon [11]. Considering the soot formation and the volume reduction from the orange juice extract, the orange peel extract was proceeded for further optimization. The orange peel also exhibited a more stable structure and comparatively less soot production after the heat treatment. This could be due to the presence of a combination of polysaccharides, cellulose, pectin, flavonoids and organic acids like citric acid which are present in the orange peel that can lead to uniform carbonization and nucleation process which proves beneficial in carbon dots production [12]. Orange peel extract was utilized for additional optimization research targeted at boosting the production and fluorescence characteristics of carbon dots because orange juice extract was determined to be inappropriate for further investigation due to severe soot production and significant volume loss.

III.2 ORANGE PEEL EXTRACT AT 160°C AND 140°C

Initially the orange peel extract was heated to 160 °C and 140 °C to determine the ideal temperature for synthesis of carbon dots. A mild brown hue was observed under normal visible light after heating step which indicates the presence of carbonization. Blue fluorescent light was observed for both the samples under UV light (365 nm) which indicates the presence of luminous carbon dots. Nevertheless, it was observed that blue emission was stronger for the sample heated at 140 °C as shown in the figure1. This indicates that nucleation of nanosized carbon structures along with uniform carbonization ensured enhanced blue emission at 140 °C [12]. The photoluminescence characteristics were improved at moderate temperature due to the creation of tiny carbon cores and functionalization of the surface by moieties like carbonyl, carboxyl and hydroxyl groups [9]. On the other hand, excessive carbonization and particle aggregation maybe formed at higher temperature of 160 °C which can lead to low emission intensity and fluorescence quenching [10]. The creation of emissive carbon nanostructures are possible because of partial carbonization and dehydration of components of orange peel such as pectin, polysaccharides and organic acids when it is heated [9]. Based on fluorescence intensity and visual qualities, the optimum temperature for synthesis of carbon dots from orange peel extract was found to be 140 °C. Therefore, for maintaining stable nanostructure development with strongest emission was found to be at this temperature.

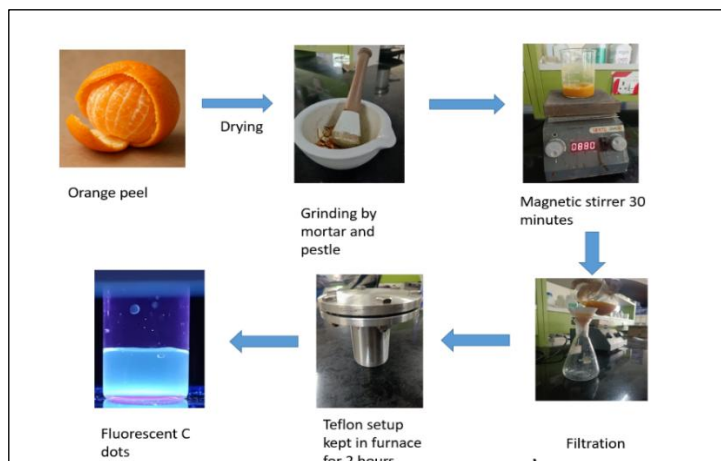


Figure 1: Green synthesis and temperature-dependent optimization of fluorescent carbon dots derived from orange peel extract.
Source: Authors, (2026).

III.3 CRYSTALLINE STRUCTURE AND MORPHOLOGY

The crystalline or amorphous nature of the material can be determined using XRD. The orange peel carbon dots show peak 24.31° corresponding to the (002) plane as shown in Fig.2. Broad peaks were detected in the XRD patterns, indicating the amorphous characteristic of the carbon dot, as confirmed by the analysis. From Fig.3, we can see that the carbon dots have a spherical-like structure. This result supports the graphitic nature of our CQDs and is consistent with the structural characteristics seen in many previous carbon dot investigations [13]. The internal structural morphology of the orange peel-based carbon dots was analysed using TEM. Therefore, the distinct peaks in the spectrum provide important data about the elemental composition of the material under analysis. EDX spectrum shows the carbon dots have high carbon and oxygen content.

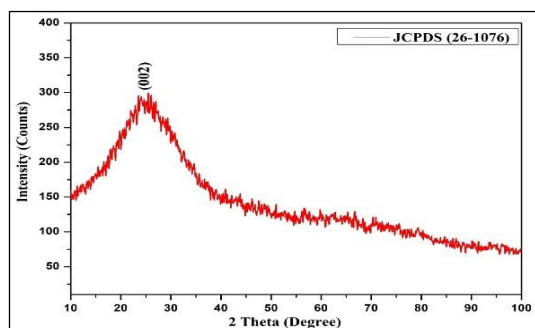


Figure 2: XRD analysis for orange peel derived carbon dots.
Source: Authors, (2026).

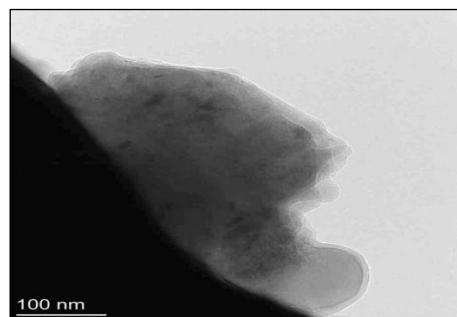


Figure 3: High-resolution TEM images for orange peel derived carbon dots.
Source: Authors, (2026).

III.4 PRESENCE OF AROMATIC STRUCTURE

The UV-visible absorption of carbon dots exhibits a peak at 230 nm as shown in Fig.4. Even though there are three peaks in the graph, with particular emphasis on the peak observed at 230 nm due to its highest intensity. This dominant peak signifies a significant absorption event attributed to the transition between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), known as the π - π^* transition. This transition is characteristic of conjugated systems, indicating the presence of aromatic structures within the carbon dots, likely stemming from C=C bonds. The smaller shoulder has a peak nearly at 320 and 280nm which represents the n- π^* transition due to C=O bonds (Table 1).

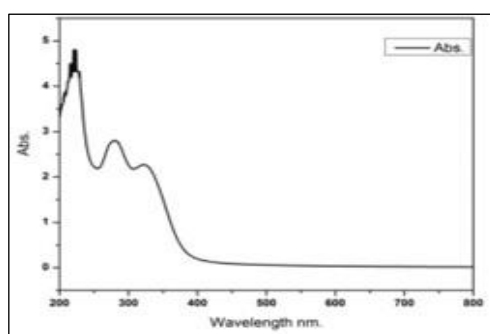


Figure 4: UV Vis absorption spectrum for orange peel derived carbon dots.
Source: Authors, (2026).

Table 1: Significance of different peaks in UV spectrum of orange peel derived carbon dots.

Peak	Wavelength (nm)	Significance
1.	270	$\pi-\pi^*$ transition of aromatic C=C bonds (graphitic core of carbon dots)
2.	340-360	$n-\pi^*$ transition of C=O groups (surface functionalization, carbonyl/carboxyl groups)
3.	400- 500	Surface defect states and functionalized groups responsible for fluorescence

Source: Authors, (2026).

III.5 IDENTIFICATION OF FUNCTIONAL GROUPS IN CARBON DOTS

The FTIR spectrum of the synthesized carbon dots offers a detailed glimpse into their molecular composition and structural characteristics. The presence of C-C stretching vibrations which is associated with aromatic backbone of carbon-based materials is indicated by the peak at 603.74 cm^{-1} . This indicates the presence of conjugated aromatic structure in the core system of the carbon dots. The C-O stretching vibrations which corresponds to the presence of oxygen containing functional groups probably from hydroxyl (-OH) or ether (C-O-C) moieties is suggested by the peak at 1066.47 cm^{-1} [12]. Additionally, the C-H bending vibrations indicating the presence of aliphatic hydrocarbon chains or methyl groups is represented by the peaks at 1250.21 cm^{-1} and 1384.26 cm^{-1} that can lead to the overall structure and stability of the CDs [8].

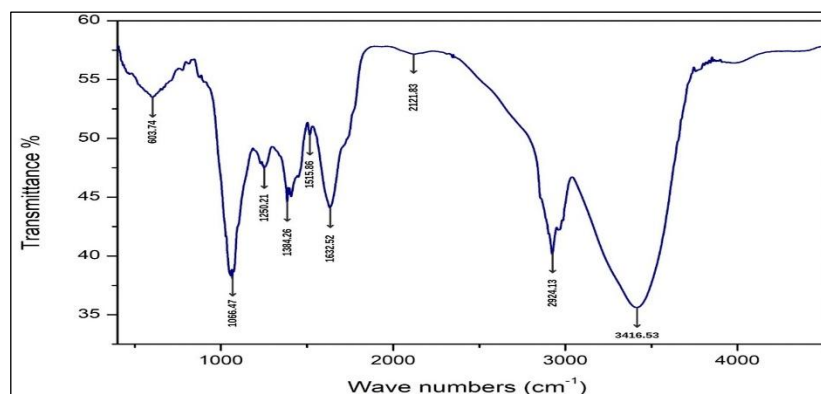


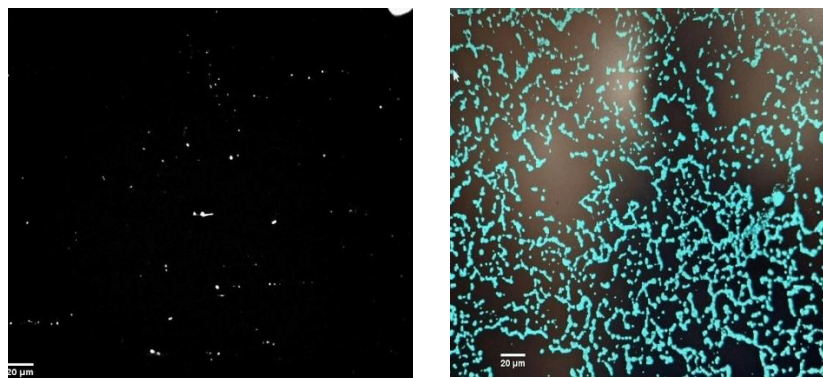
Figure 5: FTIR spectrum for orange peel derived carbon dots.

Source: Authors, (2026).

The most significant peak at 1515.86 cm^{-1} shows C=C stretching vibrations that suggest the presence of aromatic structures in the carbon dots. This indicates that the electronic and optical properties like fluorescence can be influenced by the presence of high degree of π - conjugation in the carbon dots [12]. The C=O stretching vibrations corresponds to the peak at 1632.52 cm^{-1} which indicates the presence of carbonyl groups suggestive of surface functionalization or oxidation process that took place during synthesis process. However, the peak at 2121.83 cm^{-1} could indicate unique structural features such as carbon-carbon triple bonds (C≡C), even though it might require further research. The peaks at 2924.13 cm^{-1} and 3416.53 cm^{-1} correspond to C-H and O-H stretching vibrations, respectively, signifying the presence of aliphatic hydrocarbon chains and hydroxyl groups, which are crucial for elucidating the surface chemistry and reactivity of the carbon dots (Fig. 5) [11], [12].

III.6 BACTERIAL CELLULAR UPTAKE OF CARBON DOTS

The *Staphylococcus aureus* and *Klebsiella pneumoniae* which were incubated with the orange peel-based carbon dots show a bright cyan color when visualized under fluorescence microscope as shown in Fig.6 (a,b) and Fig.7 (a,b). However, in images 6a and 7a, it was challenging to visualize the cells as they do not have dye. Hence, the synthesized carbon dot assists in the visualization of microbial cells under fluorescence microscope. Fluorescence microscopy of *Klebsiella pneumoniae* treated with orange peel-derived carbon dots showed fluorescence localized to bacterial cells. The fluorescence pattern indicated that CDs were (adsorbed onto the bacterial surface/internalized within cells), suggesting efficient interaction between CDs and the bacterial cell wall."

Figure 6: Fluorescence microscopic image of *S. aureus* (a) *S. aureus* without carbon dot (b) *S. aureus* treated with carbon dots.

Source: Authors, (2026).

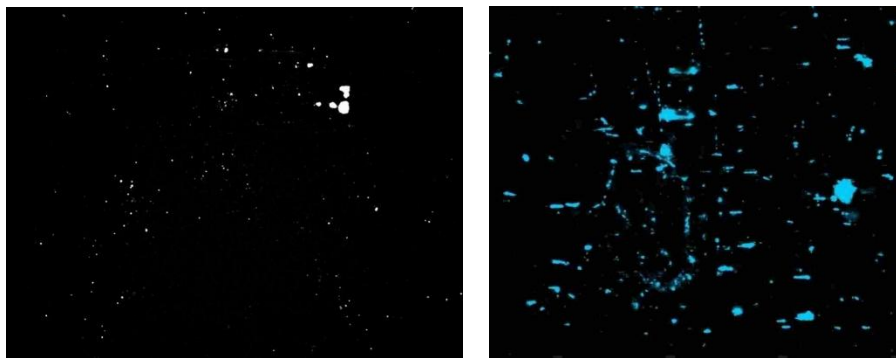


Figure 7: Fluorescence microscopic image of *K pneumoniae* (a) *K pneumoniae* without carbon dot (b) *K pneumoniae* treated with carbon dots.

Source: Authors, (2026).

III.7 DNA-CARBON DOT COMPLEX FORMATION

Using agarose gel electrophoresis, the capacity of carbon dots made from orange peel extract to interact with DNA was assessed. The first and second wells from Fig.8 indicate the loading of the DNA-Carbon dot complex, yet its presence remains invisible while the fourth and fifth wells were control groups having a loading dye. We were not able to see the DNA-Carbon dot complex due to a lack of complex formation, likely from the repulsive interaction between the two molecules. When compared to the control DNA sample, the electrophoretic mobility of DNA incubated with different carbon dot concentrations did not exhibit any discernible retardation. This implies that the produced carbon dots did not form stable interactions with DNA under the studied experimental conditions. A weak or insignificant electrostatic contact between the carbon dots and DNA molecules is shown by the lack of band shift (Figure 8).



Figure 8: Agarose gel to confirm carbon dot and DNA interaction.

Source: Authors, (2026).

III.8 DISCUSSIONS

CNPs were successfully synthesized using orange peel and orange juice extract, as demonstrated by visual inspection and a number of characterisation methods. CNPs synthesized by orange peel were given for further optimisation based on the soot formation. The presence of carbon dots was confirmed by the bright blue emission of the samples when placed in UV radiation. In this experiment, the citrus extract from the orange peel was heated at three different temperatures 200°C, 160°C and 140°C. The temperature in which the extract is heated is correlated with the emission of the carbon dot. At the right and optimized temperature, the carbon dot will be carbonized properly having a stable small size and brighter glow. From the bright glow observed on the orange peel heated at 140°C is the optimized temperature for carbon dots preparation from orange peels. Furthermore, the characterization results of FTIR, TEM, XRD and UV-visible spectroscopy have confirmed that carbon dots were fabricated from orange peel. UV-visible spectroscopy revealed a prominent absorption peak at 230 nm that confirms the optical properties of the CNPs as shown in Fig.4. Since most carbon dots have a strong π - π^* transition within their carbon structure, which is often seen as the primary absorption band in their UV-Vis spectra, a UV absorption peak around 220 nm for carbon dots usually indicates the presence of these carbon nanoparticles. XRD analysis displayed the amorphous nature of the CNPs synthesized with orange peel extract. From the FTIR results, the carbon dots have an aromatic chain backbone with various bonds attached to it [10]. The synthesized carbon dot has the ability to stain both *Klebsiella pneumoniae* and *Streptococcus aureus* cell culture. Due to their extremely tiny size, carbon dots have the potential to enter bacterial cells since particles smaller than 10 nm may be able to do so by endocytosis. The presence of oxygen as well as the small size of carbon dots makes it easy to penetrate through the cell membrane. The electrostatic attraction between the surface charge of the carbon dot and the cell membrane facilitates the endocytosis process [13]. They are also good candidates for fluorescence imaging due to their luminous qualities. Once the carbon dot passes through the cell membrane it can easily stain the cytoplasm blue color. The synthesized carbon dot stains the entire cell population in a nonspecific manner. While studying the interaction between DNA and the synthesized carbon dot there was no complex formation. The lack of observable DNA-carbon dot complex formation may be attributed to several factors. The surface charge and functional groups present on carbon dots synthesized from orange peel may not provide sufficient electrostatic attraction for the DNA as they are negatively charged.

Both DNA and the carbon dot carry negative charges, their mutual electrostatic repulsion prevents effective binding and the formation complex. Previous studies have reported that effective DNA binding typically requires positively charged surface functionalization such as amine groups. Since the synthesized carbon dots were derived from a biomass precursor without additional surface modification, the interaction with DNA may be limited. In the future experiments, the carbon dot should be doped with chemicals such as PEG or nitrogen-based molecules in order for the interaction to take place. Over time, CDs gained exceptional qualities for a range of uses due to their easy surface preparation and modification, biocompatibility, low cytotoxicity, strong resistance to photobleaching, and high-water solubility, which enables homogenous sensing tests. The observed physicochemical properties and minimal DNA interaction highlight the ability of carbon dot nanoparticles derived from orange-peel as promising candidates for future biosensing and diagnostic applications.

IV. CONCLUSION

Fluorescent carbon dot nanoparticles were hydrothermally obtained from orange peel. The fluorescence emission from the as-synthesized carbon dots was optimized by heating the orange peel extract at different temperatures and the maximum emission was attained at 140°C. Further characterizations such as XRD, Raman spectroscopy, TEM, UV-visible spectroscopy, and FTIR spectroscopy were employed to get a better understanding of the synthesized carbon dot. From this analysis we were able to understand the structure, bonds and the amorphous structure of the synthesized orange peel-based carbon dots. Carbon dots were then used to visualize pathogens. Both *Klebsiella pneumoniae* and *Streptococcus aureus* were successfully stained by carbon dots when visualized under fluorescence microscopy. The current work demonstrating the production of CNPs utilizing orange peel offers insightful results although it has limitations. It might be difficult to scale up synthesis techniques from lab to industrial production, and it could affect efficiency or quality. Since NPs may deteriorate or change over time, long-term stability in biological contexts necessitates careful research. To guarantee safety, antimicrobial activity must be confirmed in vitro and then evaluated for cytotoxicity and biocompatibility in vivo.

V. AUTHOR'S CONTRIBUTION

Conceptualization: Lakshmi S, J Sandhya.

Methodology: Lakshmi S, J Sandhya.

Investigation: Lakshmi S.

Discussion of results: Lakshmi S, J Sandhya.

Writing – Original Draft: Lakshmi S.

Writing – Review and Editing: Lakshmi S, J Sandhya.

Resources: Lakshmi S.

Supervision: J Sandhya.

Approval of the final text: Lakshmi S, J Sandhya.

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