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GREEN SYNTHESIS AND CHARACTERIZATION OF CARBON DOTS FOR EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES

Neha Rawat [1], Pratishtha Raturi[1], Nabeel Ahmad[2]

[1]Ph.D Scholar, Department of Microbiology, School Of Allied Sciences, Dev Bhoomi Uttarakhand University Dehradun, Uttarakhand
[2]Department of Biotechnology, School Of Allied Sciences, Dev Bhoomi Uttarakhand University Dehradun, Uttarakhand, Email: nabeel.biotech@gmail.com

ABSTRACT

In recent years, carbon dots (CDs) have garnered considerable attention as a promising material for biomedical applications because of their unique optical and biological properties. In this study, an eco-friendly, cost-effective and fast microwave method of preparation of carbon dots was employed. Stable C-dots were prepared from organic waste of Peels of Galgal (Citrus pseudolimon), Peels of Malta (Citrus sinensis), Leaves of Pineapple (Ananas comosus) & Leaves of Strawberry (Fragaria × ananassa) which is an economical source for the production of fluorescent carbon dots (C-dots). The development of a reddish-brown solution and blue fluorescence under UV light demonstrated the successful green synthesis of carbon dots. The generation of spherical, surface-functionalized carbon dots with average particle sizes ranging from 51.863 to 75.380 nm and unique optical properties was confirmed by UV-Vis, FTIR, HRTEM, FESEM, and fluorescence investigations. The different biological applications of C-dots such as anti-bacterial activity against *Staphylococcus aureus & Escherichia coli* and anti-fungal activity against *Candida tropicalis & Aspergillus niger* were evaluated. The synthesized carbon dots displayed specific antibacterial and antifungal properties, underlining their potential for biological and antimicrobial applications.

KEYWORDS: *carbon dots, waste, characterization, antimicrobial, peels, leaves.*

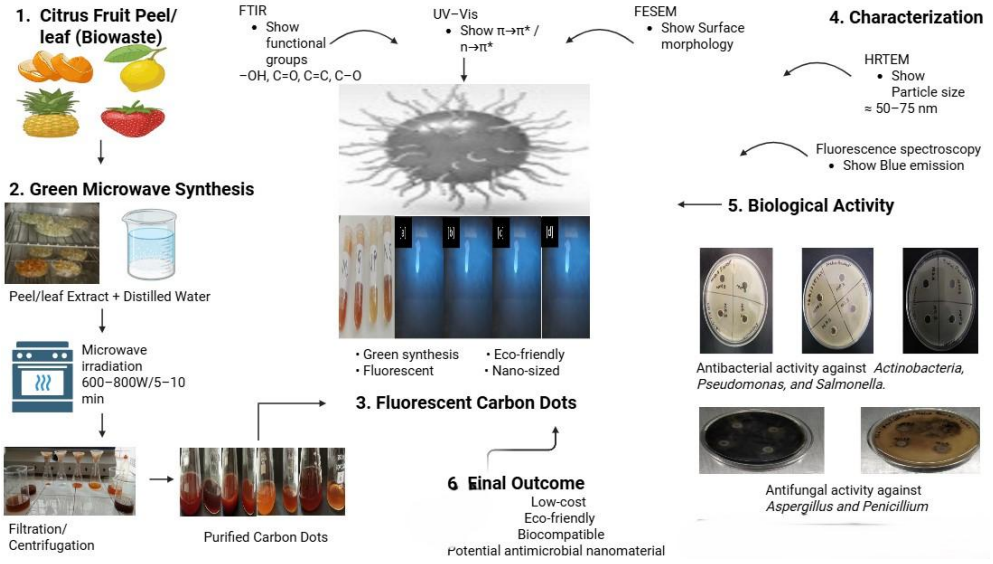


Figure 1: Shows Graphical abstract

1. INTRODUCTION

Nanotechnology is one of the emerging sciences which is applied in many sectors. Due to the nanoscale size of the materials, it is widely applicable. Nanotechnology, introduced as a concept by Feynman (1959) and termed by Taniguchi (1974), has rapidly advanced with improvements in electron microscopy. Its nanoscale materials enable wide applications across sectors, involving organic nanomaterials (nanofibers, nanogels, micelles) and inorganic ones like gold nanoparticles, quantum dots, and carbon nanotubes [1,2] In recent years, carbon dots—zero-dimensional nanoparticles—have gained significant attention due to their excellent properties like biocompatibility, low toxicity, water solubility, and high quantum yield [3]. Their ease of functionalization and broad applicability have made them a focus for many researchers. The choice of carbon precursor plays a crucial role in their synthesis, with natural and waste-derived materials, especially bio-waste, emerging as promising, eco-friendly, and cost-effective sources. This approach not only offers environmental benefits but also transforms low-value waste into valuable nanomaterials, with examples including eggshells, rice husks, lemon peels, fruit & vegetable peels, tea leaves, etc [4]. Due of their special qualities and uses, carbon dots (CDs) have become one of nanotechnology's most prized gifts. Typically, CDs are carbon nanoparticles with

an average diameter of less than 10 nm[5]. CDs were discovered accidentally in 2004 at the time of purification of single wall carbon nanotubes (SWCNTs) by Xu et al. [6]. Two years later, in 2006, Sun et al. first synthesized stable photoluminescent carbon nanoparticles of different sizes and named them "carbon quantum dots" (CQDs) [7]. Carbon dots (CDs) are the preferred term for fluorescent carbon nanoparticles that have a diameter of less than 10 nm. Carbon dots (CDs), <10 nm carbon-based nanomaterials discovered in 2004, have gained attention for applications in sensors, inks, catalysts, drug delivery, and LEDs due to their excellent optical and structural properties. Compared to traditional quantum dots, CDs offer advantages like tuneable emission, biocompatibility, eco-friendliness, and high solubility. They can be synthesized via top-down or bottom-up methods, with hydrothermal treatment favoured for its simplicity and low energy use. Using plant-based precursors rich in functional groups enhances CD properties without needing toxic chemicals or surface passivation. Hence, natural carbon sources remain a key focus for eco-friendly CD synthesis [8]. To bridge the gap left by insufficient advancement and investigation, researchers are now emphasizing the biological uses of CDs, such as their anti-inflammatory and antibacterial properties. In addition, compared to metal CDs, organically derived CDs are less hazardous. Thus, it is important to develop CDs as a possible biological

application option. Research on using natural materials to synthesize carbon dots (CDs) gained momentum in 2012 after the first study using grass. Biomass offers hydrophilicity and self-passivation due to its rich organic content, making CD synthesis simpler and more cost-effective than chemical methods. CDs have been produced from diverse biomass sources, including fruits, vegetables, food waste, plant parts, animal derivatives, and industrial byproducts [9].
Food is vital for sustainable living, but rising populations increase food waste. According to UNEP, around 1.05 billion tonnes of food waste were generated globally in 2022—19% of available food. Fruits alone account for 45% of this waste. Food waste is the third-largest source of greenhouse gas emissions, contributing 4.4 Gt CO_2 eq/year [10]. Carbon dots (CDs) from fruit waste offer an innovative way to reduce environmental waste. Rich in nutrients, fruit waste is a valuable yet underused resource [11,12]. Fruit waste is a cheap, abundant, eco-friendly alternative to synthetic carbon sources for CD production [13]. CD synthesis from biomass is simpler and cheaper than chemical methods. Sources include fruits, vegetables, food waste, animal and plant derivatives, and industrial byproducts [14]. Fruit waste was chosen as it's widely consumed and can contribute 10–70% of environmental waste, depending on usage [15,16].
In the current work, we concentrated on creating luminous CDs from four distinct household wastes: peels of Galgal [NG], peels of Malta [NM], leaves of pineapple [NP], and leaves of strawberry [NS] using a straightforward, greener, economical, and environmentally friendly method. Also, an inexpensive microwave approach was chosen to synthesize CDs . This study also demonstrates the innovative 'green solution' to dispose of environmental waste. The synthesized CDs were characterized by numerous techniques like Spectroscopic methods (UV–Vis Spectroscopy, FTIR, Fluorescence Spectroscopy) and microscopic methods (HR-TEM, FE-SEM). The anti-bacterial and anti-fungal properties of biosynthesized carbon dots were further studied for biopharmaceutical usage.

2.MATERIAL & METHOD

2.1.Biosynthesis of Carbon dots from Fruit Waste

2.1.1.Chemicals and Reagents

All chemicals and reagents [0.1 N NaOH, PEG 200] utilized in this investigation were of high analytical grade and were available in the school of pharmacy and research laboratory of the college premises (Dev Bhoomi Uttarakhand University, Dehradun). Deionized water was employed in all the experiments, until stated otherwise. Fresh sample i.e. Peels of Galgal & Peels of Malta were gathered from the vicinity of Tehri Garhwal, Uttarakhand and Leaves of Strawberry & Leaves of Pineapple were bought from the nearby market of Dehradun, Uttarakhand. Nutrient broth, MH agar, nutrient agar, potato dextrose agar, acetone, 70% ethanol, etc used in this study were of high analytical grade and were available at Rebioin Biotech labs, Dehradun, Uttarakhand.

2.1.2.Micro-organisms

Bacterial cultures (*Staphylococcus aureus & Escherichia coli*) and Fungal culture (*Candida tropicalis & Aspergillus niger*) were available at Rebioin Biotech labs, Dehradun, Uttarakhand.

2.1.3.Preparation of Peel/ Leave Extract

Fresh peels and leaves of NG, NM, NP, NS were picked to prepare C-dots. The peels/ leaves were cleaned with running tap water to get rid of the dust. Peels/ leave were then chopped into tiny pieces and fully dried in hot air at a temperature of more than 65 °C. Once drying is complete, the peels/ leaves were powdered with a mixer grinder. For use in subsequent procedures, this powder can be kept for a month. 250 mL of distilled water was filled in a round-bottom flask and 6.25 g of powder added to it . The round-bottom flask is placed on the heating mantle and heated at 70 °C for roughly 40 minutes. After the mixture reaches room temperature, it is centrifuged for ten minutes at 10,000 rpm. The peel/leave extract is the term used to describe the collected supernatant. C-dot preparation is another usage for this. This can be kept below 5 °C for no more than three days [17]. This process is done for all the 4 samples separately.

2.1.4.Synthesis of Carbon dots

C-dots were synthesized using the NG, NM, NP, and NS extracts. The microwave technique is used for the synthesis. For synthesis, the obtained extracts of NG, NM, NP, and NS (50 mL each) are combined with 1mL each of 0.1 N NaOH and polyethylene glycol (PEG) 200. Microwave radiation is applied to this solution for two minutes at intervals of ten seconds each [microwave irradiation 600–800W/5–10 min]. When lengthy chains of carbohydrates are exposed to microwave

radiation, the chain breaks down at the nanoscale, creating what are known as C-dots. This is left to cool until it reaches room temperature. The C-dots synthesized can be stored at 4 °C [17].

2.2.CHARACTERIZATION OF THE BIOSYNTHESIZED CARBON DOTS

To study the absorption and emission peaks of C-dots, the characterization was done by UV-Vis Spectrophotometer, Fluorescence Spectroscopy and functional group identification through FTIR and particle size determination from HR-TEM and the presence of C-dots was also confirmed by FE-SEM.

2.2.1UV-Vis Double Beam Spectroscopy (UV- VIS SPECTROMETER)

The CDs samples were analysed in the range of scanning 200– 600 nm with resolution being 1nm, using Cary 60 UV-Vis Spectrophotometer Agilent Technologies instrument. The experimental absorption spectrum of compounds in the 200–600 nm regions in unpolarized light was obtained by using a UV-visible spectrophotometer. The estimated and observed absorption spectra demonstrate the accuracy of the derived model calculations. UV-Vis analysis was carried out at CytoGene Research & Development, Lucknow, Uttar Pradesh.

2.2.2Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (FTIR)

CDs samples were analysed using potassium bromide pellet method. The pellets were exposed to IR waves in the range of 400–4000 cm−1 to acquire the spectra. Perkin-Elmer Spectrum Two with Universal ATR was used to record FTIR at the temperature of room . FTIR analysis was aimed at the recognition of bio-functional groups attached to the nanoparticles. The FTIR study was performed at CytoGene Research & Development, Lucknow, Uttar Pradesh.

2.2.3High Resolution-Transmission Electron Microscopy [HR-TEM]

The morphology and particle size of the produced CDs were examined using HR-TEM. HR-TEM was recorded with a JEM 2100 plus, Model: JEOL instrument operated at 80- 200 kV. Copper grids coated with carbon were covered with the diluted solution, which was then allowed to dry outside. Ten sites on the TEM grid were analyzed in total. HRTEM analysis was carried out at Centre for Instrumentation and Laboratory Facilities, Panjab University Chandigarh.

2.2.4Fluorescence Spectroscopy (SPECTRO-FLUOROMETER)

A JASCO Japan, Model : FP-8550 fluorescence spectrometer was equipped to obtain the fluorescence spectra as it has a high sensitivity measurement due to a design that prohibits fluorescence detecting along the flow channel. Fluorescence Spectroscopy analysis was carried out at Sophisticated Analytical Instrumentation Facility lab, Panjab University Chandigarh.

2.2.5Field Emission Scanning Electron Microscopy (FE-SEM)

Field emission scanning electron microscopy Hitachi, Japan Model: SU 8010 Series was used to determine the surface morphology of CDs. FE-SEM analysis was carried out at Sophisticated Analytical Instrumentation Facility lab, Panjab University Chandigarh.

2.3Anti-bacterial Activity of C-dots

The evaluation of antimicrobial activity of C-dots synthesized by peel/ leave extract was tested against *Staphylococcus aureus & Escherichia coli* strains of clinical isolates. The anti-bacterial activity was carried out by well diffusion method. The well diffusion assay is regarded as one of the best techniques for the study of antibacterial susceptibility[18]. The antibacterial activity of C-dots was tested against 2 clinical isolates were performed at Rebioin Biotech labs, Dehradun, Uttarakhand.

2.4Anti-fungal Activity of C-dots

The evaluation of antifungal activity of C-dots synthesized by peel/ leave extract was tested against *Candida tropicalis & Aspergillus niger*. The anti- fungal activity was carried out by well diffusion method. The well diffusion assay is regarded as one of the best techniques for the study of antifungal susceptibility. For the study of anti-fungal activity, potato dextrose agar was used [19, 20]. The antifungal activity of C-dots was tested against 2 clinical isolates were performed at Rebioin Biotech labs, Dehradun, Uttarakhand.

3.RESULT & DISCUSSION

3.1.Biosynthesis of Carbon dots from Fruit Waste

The microwave irradiation method of C-dots synthesis was carried out for 2 min at an interval of 10 s each. This resulted in the change of the colour to reddish brown shows formation of C-dots (fig 2). Initial studies were conducted using the UV chamber to verify the creation of carbon dots. UV light (354 nm) was directed onto the tube holding the carbon dot solution while the samples were in the UV chamber. The presence of carbon dots was verified by a blue fluorescence observed at 354 nm (fig 3). Ordinary solution was found to exhibit no fluorescence when exposed to ultraviolet light.

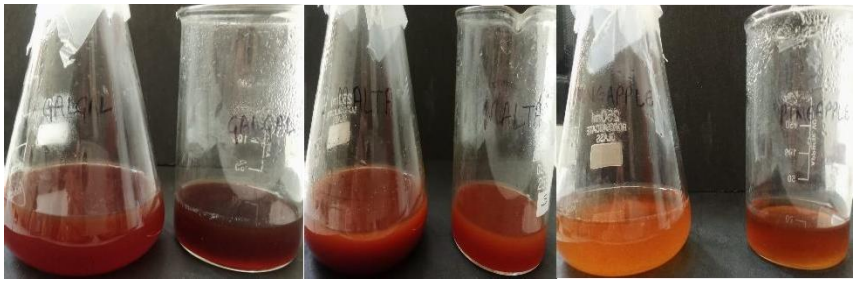


Figure 2: Change in color to reddish brown shows the formation of CDs [a]NG [b]NM [c]NP [d]NS

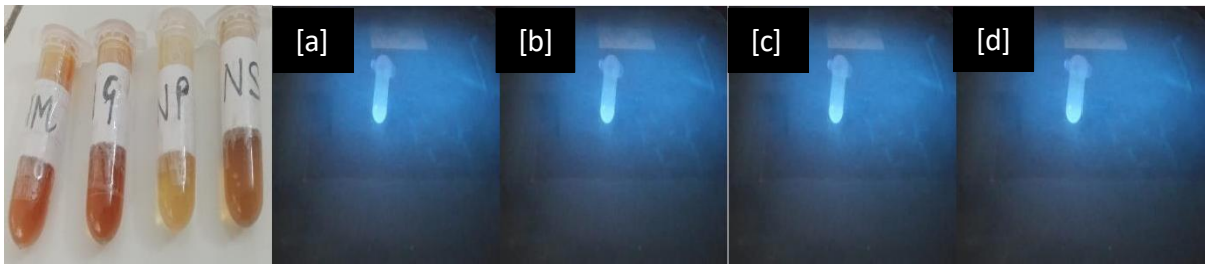


Figure 3: Solution showing fluorescence in UV light confirming the biosynthesis of carbon dots [a]NG [b]NM [c]NP d]NS

3.2.Characterization of the biosynthesized Carbon dots

3.2.1.UV-Vis Double Beam Spectroscopy (UV- VIS SPECTROMETER)

Multiple significant peak in UV and visible region was observed. 210–280 nm: Strong $\pi \rightarrow \pi^*$ transitions of sp^2-hybridized C=C bonds in the graphitic carbon core. 290–360 nm: $n \rightarrow \pi^*$ transitions arising from C=O, –COOH, and other oxygen-containing surface functional groups. 360–470 nm: Broad absorption due to surface defect states, surface oxidation, heteroatom doping, and extended π-conjugation, which contribute to the optical and fluorescence properties of carbon dots. The results for NG, NM, NP, NS are shown in (fig 4).

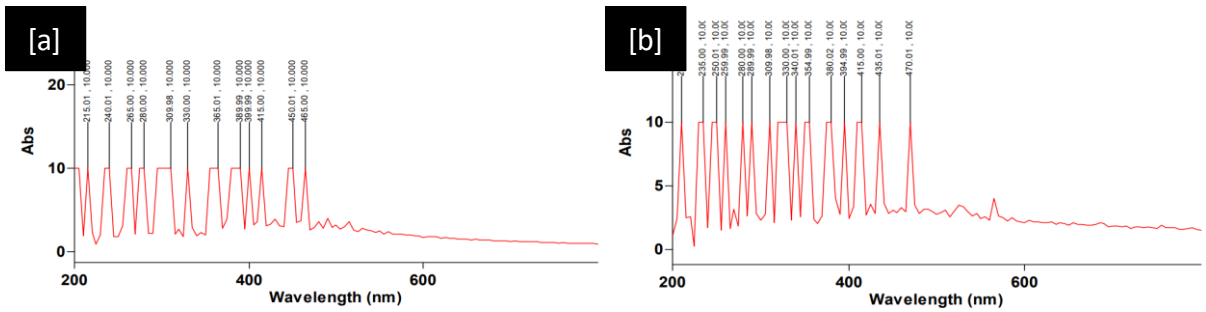
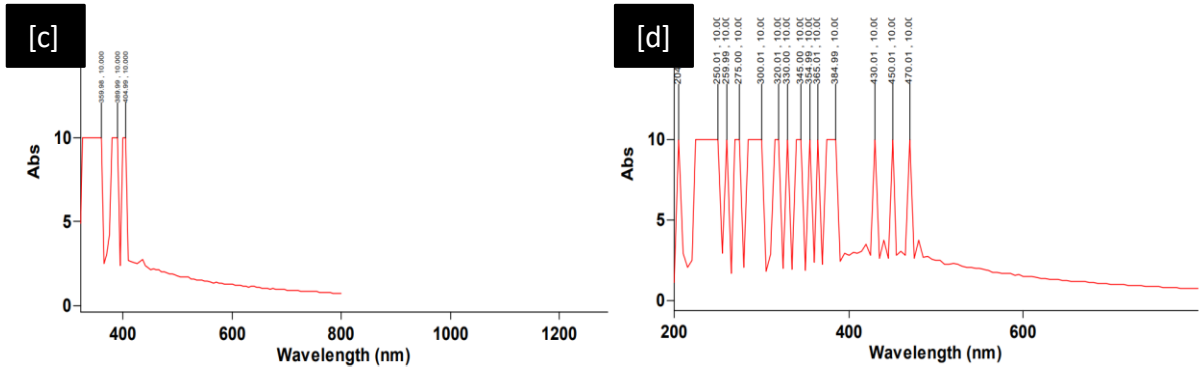


Figure 4: UV- Vis graph showing electronic transitions present in [a]NG [b]NM [c]NP [d]NS



3.2.2.Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy [FTIR]

The FTIR spectral analysis of green synthesized carbon dots were obtained. 3300–3400 cm^{-1}: O–H stretching, confirming hydroxyl groups on the CD surface. 2900–2918 cm^{-1}: Aliphatic C–H stretching, indicating residual organic carbon chains. 1640–1740 cm^{-1}: C=O stretching, confirming carbonyl/carboxyl functional groups formed during carbonization. 1540–1610 cm^{-1}: C=C stretching, indicating graphitic or aromatic sp^2 carbon domains. 1000–1300 cm^{-1}:

C–O stretching, confirming alcohol, ether, and ester functionalities on the carbon dot surface. Fingerprint region (<1500 cm^{-1}): Contains characteristic vibrations unique to the synthesized carbon dots and their surface chemistry. The results for NG, NM, NP, NS are shown in (fig 5).

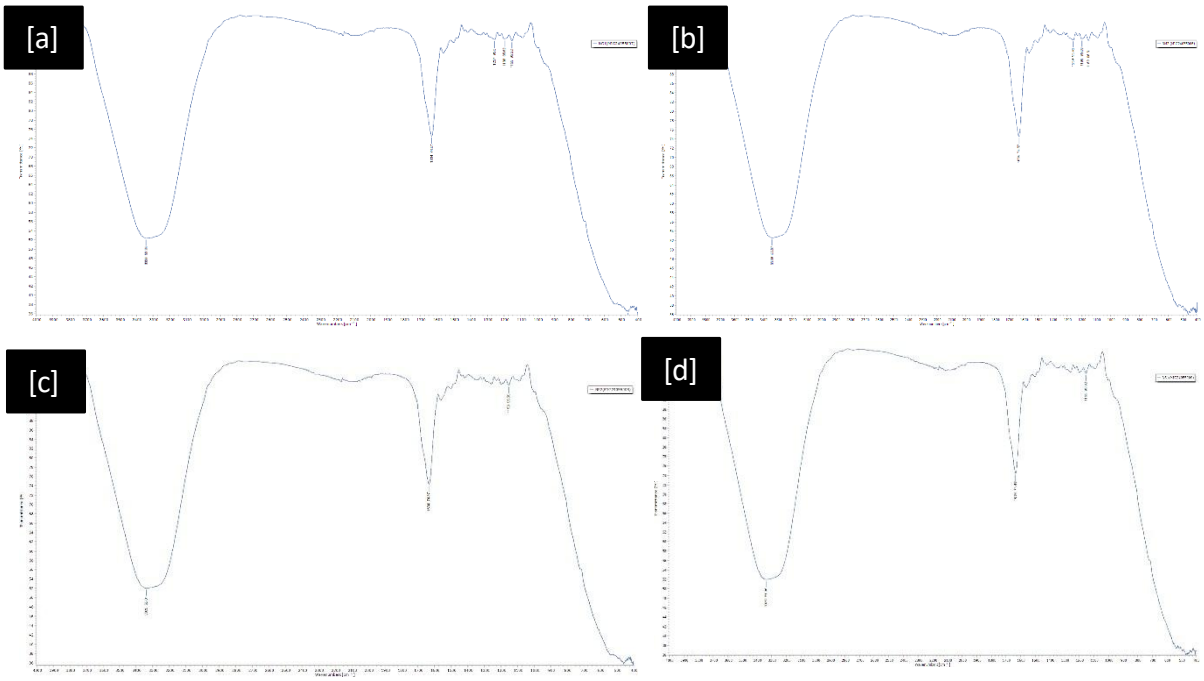


Figure 5: FTIR graph showing functional groups present in [a]NG [b]NM [c]NP d]NS

3.2.3.High Resolution-Transmission Electron Microscopy [HR-TEM]

The effective production of mostly spherical carbon nanoparticles in all four samples was verified by HRTEM analysis. 56.327 nm (NG), 75.380 nm (NM), 70.966 nm (NP), and 51.863 nm (NS) were the mean particle sizes. NP had the highest degree of agglomeration despite having the second-biggest particle size among the samples, while NM had the largest average particle size and the best particle dispersion. NS had the smallest average particle size with moderate aggregation, whereas NG was made up of moderately aggregated nanoparticles buried in an amorphous carbonaceous matrix. The results for NG, NM, NP, NS are shown in (fig 6).

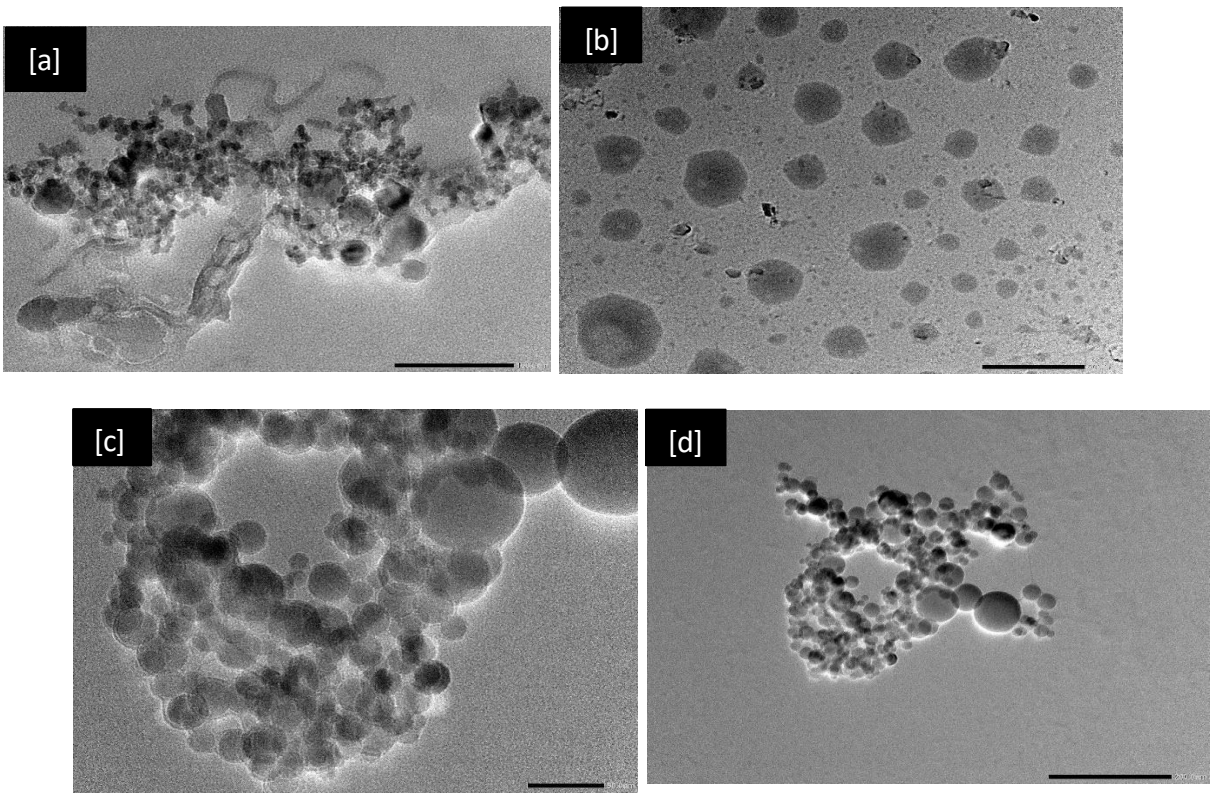


Figure 6:HRTEM images showing the morphology and dispersion of the synthesized carbon dots [a]NG [b]NM [c]NP d]NS

2.4.1Fluorescence Spectroscopy (SPECTRO-FLUOROMETER)

The four samples fluorescence spectra showed different emission characteristics, suggesting variations in their surface chemistry and optical features. At about 536.5 nm, NG displayed a strong green emission peak that suggested extremely effective and consistent emissive centres. NM showed a moderately intense broad green emission peak at 524 nm, suggesting the existence of several surface emissive states. Multiple emissive centres and heterogeneous surface chemistry are suggested by NP dual emission peaks at about 455 nm and 505 nm, which correlate to blue-cyan/green fluorescence. NS showed a broad, weak yellow-orange emission that was focused at 580 nm, suggesting considerable fluorescence quenching and deep surface defect states. Overall, the fluorescence behaviour shows that the synthesized carbon dots have distinct emissive characteristics because of differences in the degree of carbonization and surface functional groups. The results for NG, NM, NP, NS are shown in (fig 7).

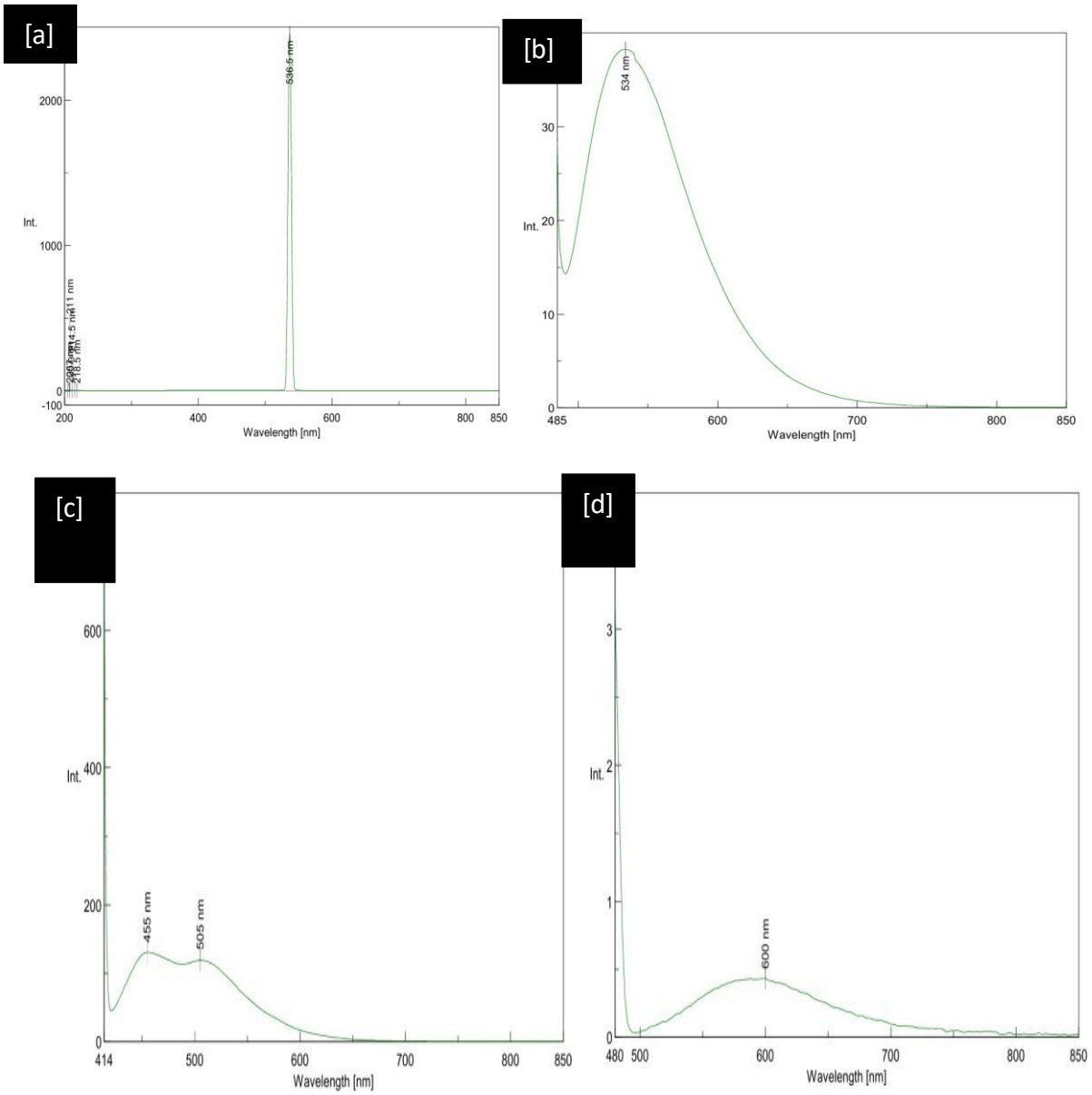


Figure 7: Fluorescence spectra showing the optical emission properties of the synthesized carbon dots [a]NG [b]NM [c]NP d]NS

3.2.4.Field Emission Scanning Electron Microscope [FE-SEM]

The four samples had different surface morphologies, but the FESEM analysis showed that the carbonaceous particles were mostly spherical. NG displayed a virtually flawless spherical particle with a smooth surface and minimal agglomeration. Amorphous carbon was present in NM, which similarly displayed a smooth spherical particle encircled by a rough granular matrix. NP showed a spherical particle with strong structural homogeneity and a little amount of surface roughness. NS featured a bigger spherical particle with a smooth surface and tiny protrusions, suggesting connected carbonaceous pieces. Overall, the FESEM pictures showed the generation of spherical carbonaceous particles with strong structural integrity and negligible aggregation. The results for NG, NM, NP, NS are shown in (fig 8).

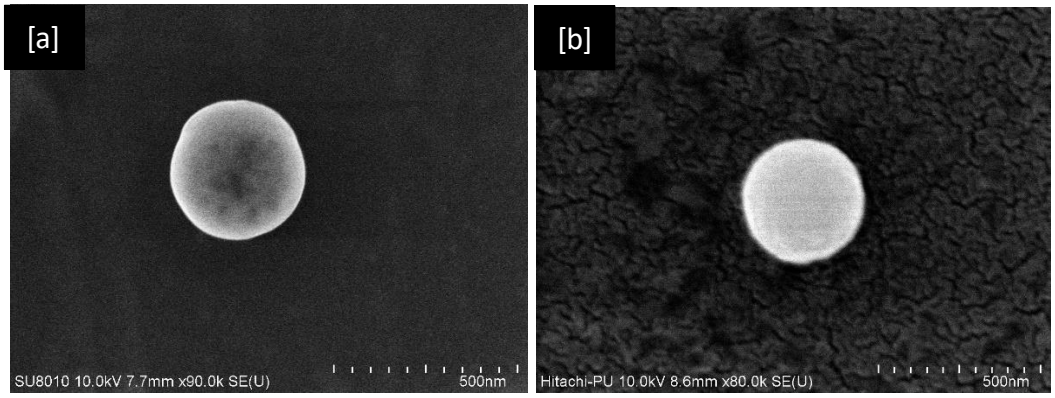
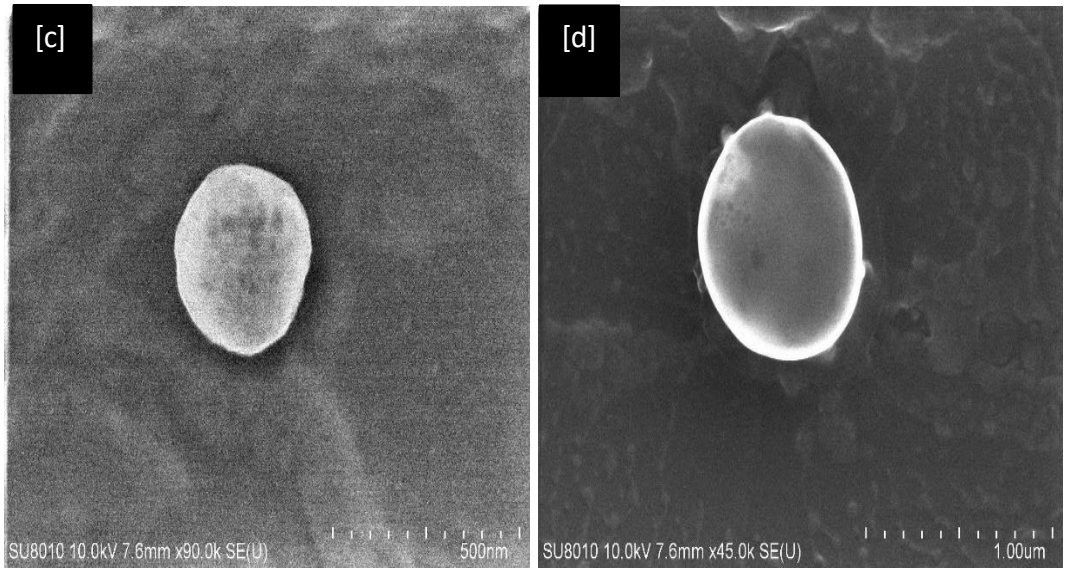


Figure 8: FESEM images showing the surface morphology of the synthesized carbon dots [a]NG [b]NM [c]NP d]NS



3.3.Anti-bacterial Activity of C-dots

Using the zone of inhibition approach, the antibacterial activity of samples NG, NM, NP, and NS was assessed against *Staphylococcus aureus & Escherichia coli*. The samples showed variable antibacterial activity, with inhibition zones ranging from 0 to 20 mm, while the standard antibiotic exhibited higher activity (15–20 mm). The control showed no inhibition, indicating that some samples possess potential antibacterial properties. Anti-bacterial activity of NG, NM,NP,NS against *Staphylococcus aureus & Escherichia coli* is shown in (fig 9 & fig 10). Zone of inhibition of NG, NM,NP,NS against *Staphylococcus aureus & Escherichia coli* is shown in (table 1 & table 2).



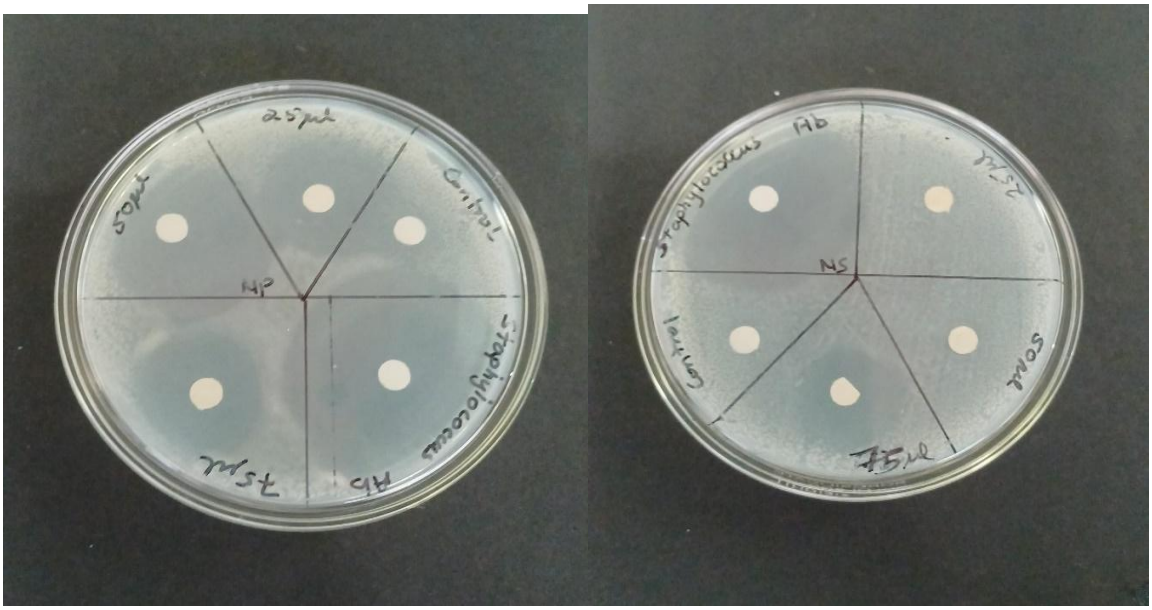


Figure 9: Showing anti-bacterial activity of NG, NM, NP, NS against *Staphylococcus aureus*

Table 1: Showing zone of inhibition of NG, NM, NP, NS against *Staphylococcus aureus*

ZOI	NG	NM	NP	NS
CONTROL	0	0	0	0
ANTIBIOTIC	19	15	19	17
25	6	4	6	0
50	9	8	11	4
75	15	11	14	11

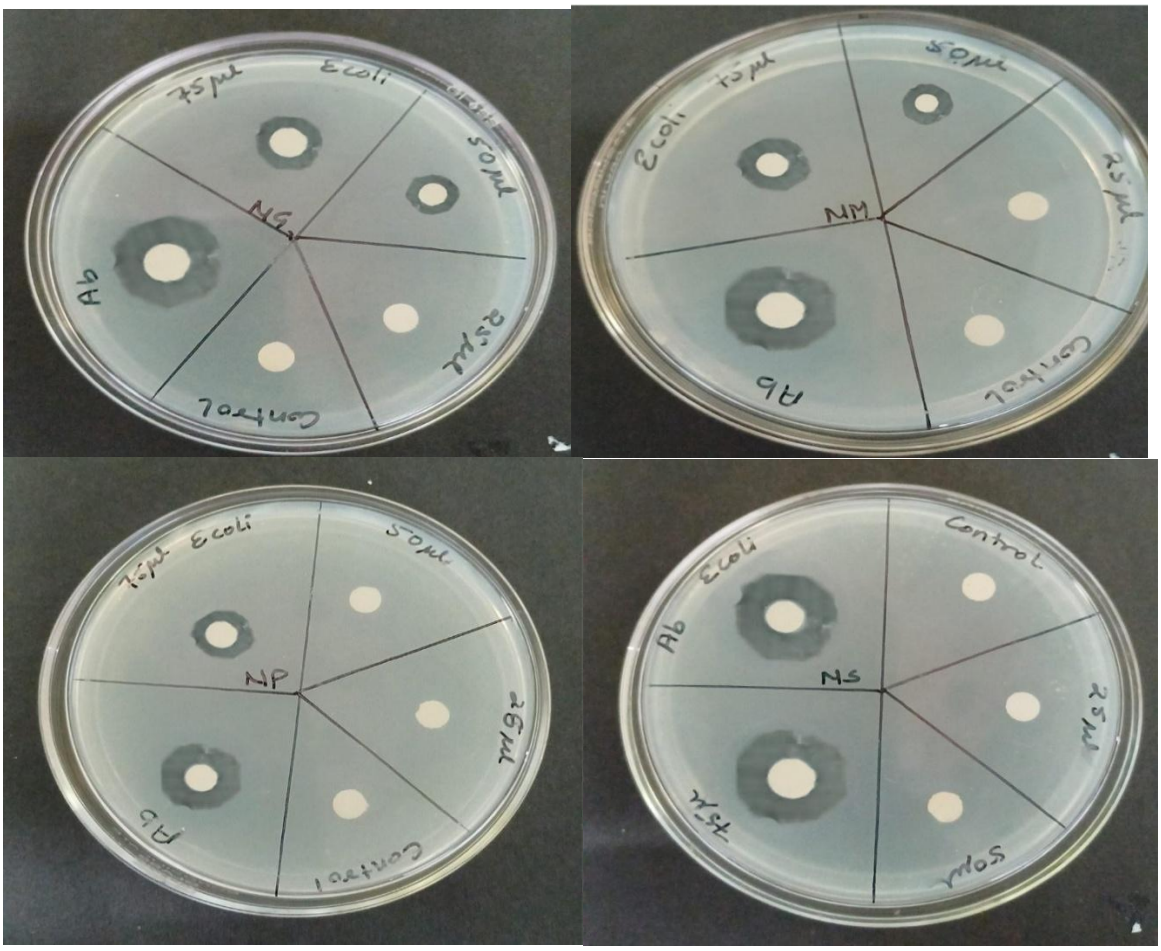


Figure 10: Showing anti-bacterial activity of NG, NM, NP, NS against *Escherichia coli*

Table 2: Showing zone of inhibition of NG, NM, NP, NS against *Escherichia coli*

ZOI	NG	NM	NP	NS
CONTROL	0	0	0	0
ANTIBIOTIC	11	10	12	11
25	2	5	0	0
50	7	6	3	7
75	10	9	9	10

3.4.Anti-fungal Activity of C-dots

The zone of inhibition approach was used to evaluate the antifungal activity of samples NG, NM, NP, and NS against *Aspergillus niger & Candida tropicalis*. The samples exhibited variable antifungal activity, with inhibition zones ranging from 0 to 10 mm, while the standard antibiotic showed higher activity (10–20 mm). The control showed no inhibition, indicating that some samples possess antifungal potential, although their effectiveness was lower than that of the standard antibiotic. Anti-fungal activity of NG, NM,NP,NS against *Aspergillus niger & Candida tropicalis* is shown in (fig 11 & fig 12). Zone of inhibition of NG, NM,NP,NS against *Aspergillus niger & Candida tropicalis* is shown in (table 3 & table 4).

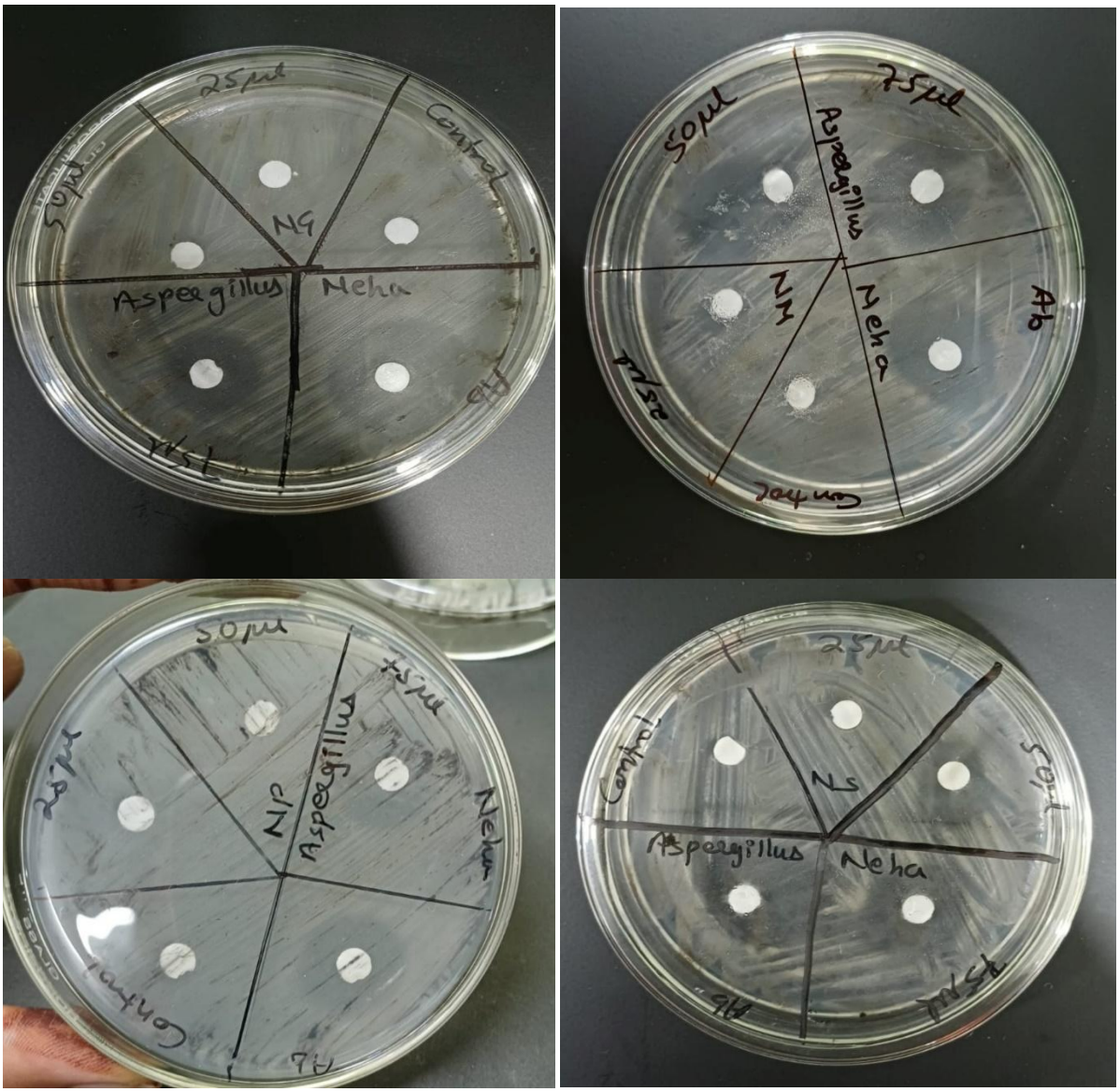


Figure 11: Showing Anti-bacterial activity of NG, NM, NP, NS against *Aspergillus niger*

Table 3: Showing zone of inhibition of NG, NM, NP, NS against *Aspergillus niger*

ZOI	NG	NM	NP	NS
CONTROL	0	0	0	0
ANTIBIOTIC	20	19	10	10
25	0	0	0	0
50	7	6	5	1
75	10	9	5	5

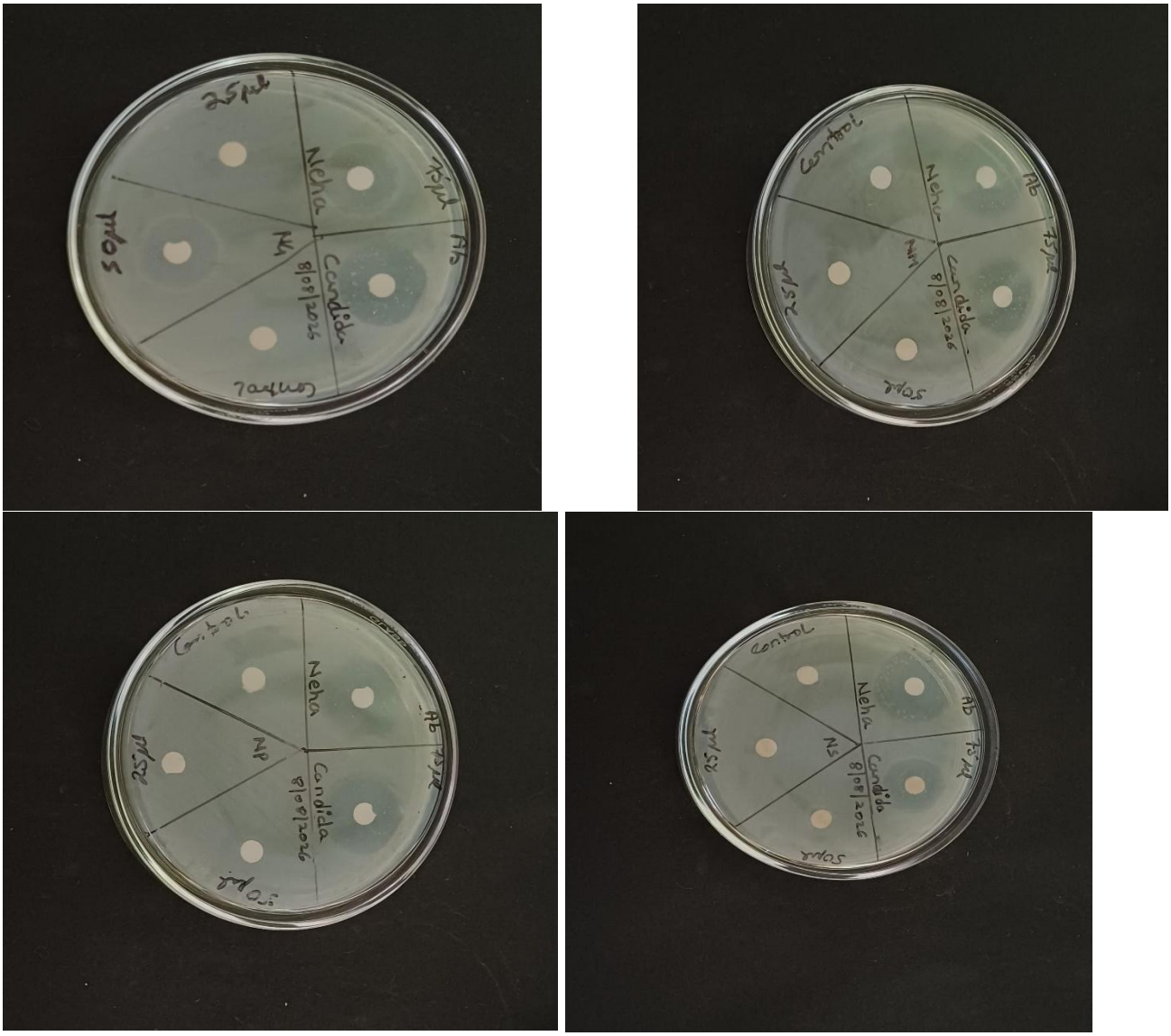


Figure 12: Showing Anti-bacterial activity of NG, NM, NP, NS against *Candida tropicalis*

Table 4: Showing zone of inhibition of NG, NM, NP, NS against *Candida tropicalis*

ZOI	NG	NM	NP	NS
CONTROL	0	0	0	0
ANTIBIOTIC	12	9	9	10
25	0	0	0	0
50	6	5	0	0
75	7	12	10	7

4.CONCLUSION

For large-scale production, the one-step micro wave synthesis method of C-dots is straightforward, easy, economical, and environmentally benign as a good supply of organic waste material with a high carbon content. The development of a reddish-brown solution and blue fluorescence under UV light demonstrated the successful green synthesis of carbon dots.

The generation of spherical, surface-functionalized carbon dots with average particle sizes ranging from 51.863 to 75.380 nm and unique optical properties was confirmed by UV-Vis, FTIR, HRTEM, FESEM, and fluorescence investigations. The synthesized carbon dots displayed specific antibacterial and antifungal properties, underlining their potential for biological and antimicrobial applications. The research expands the range of potential biomedical uses for biosynthesized C-dots.

Compliance with Ethical Standards
Conflict of Interest- The authors declare that they have no conflict of interest.

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