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Green Synthesis, Characterization, and Assessment of the Antimicrobial Activity of Metal-Free Carbon Dots Derived from *Moringa oleifera* Leaves

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Abstract

Carbon dots are fluorescent carbon nanomaterials that are less than 10 nm in size and they have gained considerable gaining importance due to their stability in chemicals, very good water solubility nature, cost effective and fluorescence properties. In this research, the extraction of carbon dots (C-dots) from the Moringa, and their potential to wield as a potent mineralized antibacterial agent, are under rigorous scrutiny. A single hydrothermal crucible was harnessed to smelt the CDs. Additionally, analytical tools like UV-visible spectrophotometry, fluorescence spectroscopy, and Fourier Transform Infrared Spectroscopy (FT-IR) were used to analyze the produced C-dots. By using the minimum inhibitory concentration and agar diffusion techniques, the antibacterial activity of the as-synthesised carbon dots was examined against Bacillus sp., Escherichia coli, Pseudomonas sp., and Klebsiella. Results suggest that the synthesised carbon dots have maximum antibacterial activity against Pseudomonas sp. These results reveal that Moringa leaves are a potential source of carbon dots with antimicrobial efficiency.

Keywords: *Antimicrobial Activity, Carbon Dots, Green Synthesis, Minimum Inhibitory Concentration, Moringa*

1.0 Introduction

Nanotechnology is a developing field of scientific and technological sectors. Globally, the field is developing tremendously quickly. Recent years have seen a significant increase in interest in nanotechnology due to its remarkable mechanical, electrical, optical, and magnetic capabilities. The concept of nanoscale devices was put forth by Richard Feynman in 1959. Norio Taniguchi first used the term "Nanotechnology" in 1974. The accelerated rate of nanotechnology in the 1980s with the discovery of electron microscopy. Organic nanomaterials, reversed micelles, nanofibers, nanogels, micelles, and nanoparticles in the form of inorganic materials, such as carbon nanotubes, gold nanoparticles and quantum dots, are among the several forms of nanoparticles[1]. Carbon Dots (CDs), a subclass of carbon nanomaterials, have attracted a lot of interest because of their desirable characteristics, including low toxicity, tiny size, water solubility, chemical stability, affordability, high electrical conductivity,

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notable biocompatibility, and photoluminescence (PL) characteristics, which were unintentionally found by Xu *et al.* in 2004 when purifying single-walled carbon nanomaterials[2]. Numerous industries, including medication delivery, diagnostics, biosensing, catalysis, and bioimaging, use carbon dots in a wide variety of ways.

The carbon dots can be synthesized by two methods, that is the top-down method and the bottom-up method. By using laser treatment, arc discharge, and electrochemical procedures to split larger carbon ores into smaller mineralized fragments, the carbon dots are created using the top-down method. In the bottom-up approach, the CDs are smelted from carbon precursors through the mineral-rich hydrothermal method and microwave-assisted pyrolysis method[3-5], ultrasonic method, pyrolysis method and acid dehydration method. However, the above-mentioned methods have certain limitations such as the use of chemicals, requiring expensive carbon sources, low product yield, and high temperature. Researchers are involved in new methods of synthesizing carbon dots[5]. According to reports, the hydrothermal approach is risk-free, straightforward, non-toxic, economical, and biocompatible.

Researchers are interested in using plant material as a carbon source for the hydrothermal synthesis of carbon dots. Ginkgo leaves[7], *Ocimum sanctum*[8], *Prunus mume*[9], sweet potato[10], sugarcane bagasse[11], radish[12], rose petals, mustard seeds[13], aloe vera[14], onion waste[15], finger millet[16], spices[17], and others are examples of plant materials that have been studied for the synthesis of carbon dots. The CDs produced using environmentally friendly synthetic methods have found widespread use in fields such as pH, temperature-responsive fluorescence sensor manufacturing for vitamin B12 identification[18,19], extra/intracellular metal ion detection, and multi-color live cell imaging[20].

In continuation of the above, *Moringa oleifera* leaves are used as a carbon source, grow in the subtropical and tropical regions of the world and native to India. Numerous studies on the antioxidant, anticancer, anti-inflammatory, and antimicrobial effects of Moringa leaves have been evaluated.

The average nanoparticle sizes were adversely correlated with the antibacterial activity. According to the studies, biologically created nanoparticles that use plant extract have greater antibacterial activity than those created chemically. Career prospects for plant-mediated nanoparticle synthesis include industrializing lab-based research, identifying the precise mechanism driving harmful bacterial suppression, and analyzing phytochemicals implicated in nanoparticle synthesis using bioinformatics techniques.

The main purpose of our examination is to yield carbon dots from *Moringa oleifer* leaves via hydrothermal method. This method is eco-friendly; it does not require any harmful or hazardous chemicals. The synthesised carbon dots are evaluated for antimicrobial efficiency.

2.0 Materials and Procedures

2.1 Materials and Microorganisms

Moringa leaves (*Moringa oleifera*) were purchased from a local market, Ethanol, nutrient agar, and nutrient broth. The antibacterial investigation included four therapeutically significant bacterial strains as test organisms. The bacterial strains gram positive Bacillus *sp.* and the gram negative bacteria Klebsiella *sp.,* Pseudomonas *sp.* and *E. coli.* The cultures were the isolates from the Department of Microbiology, MSRCASC, Bengaluru, Karnataka.

2.2 Sample Preparation

The Moringa leaves were sterilized with distilled water and then it was ground into fine paste by adding double distilled water in a grinder/mixer. This paste was added to the hydrothermal bomb. Hydrothermal synthesis is used in the preparation of nanomaterials. In this process, nanomaterials are synthesized under high pressure and temperature. The hydrothermal bomb was held in a hot air oven at 180°C for 6 hours after the extract was added to it. A hot air oven creates the required high pressure and temperature conditions. Then, it is cooled for 12 hours and taken out of the hot air oven.

2.3 Filtration of the Extract

With the aid of Whatman filter paper, the resulting extract was purified (42nm). After discarding the residue, a 10-minute centrifugation at 8000 rpm was

performed on the filtrate. The sample was centrifuged once more at 8000 rpm for 10 minutes after being cleaned by washing it three times with distilled water and ethanol. The supernatant was collected from the centrifuge tubes and it was added into a beaker. This supernatant in the beaker is used as sample for further analysis.

2.4 Study of the Antibacterial Activity of the Compound

Two approaches were used in the antibacterial study:

a. Minimum Inhibitory Concentration
b. The agar diffusion method

2.4.1 Minimum Inhibitory Concentration (MIC)

To understand the MIC of the compound, the tube dilution method was followed against the test bacterium. The bacterial strains gram positive Bacillus *sp.* and the gram negative bacteria Klebsiella *sp.,* Pseudomonas *sp.* and Escherichia coli were cultured in nutrient broth for 48 hours.

The 5ml of sterile nutrient broth was taken with 10 µl of bacterial culture and exposed to different concentrations of the synthesized carbon dots that includes 10 µl, 50 µl and 100 µl followed by higher concentrations of 200 µl, 300 µl, 400 µl, and 500 µl. The tubes were then kept at 37°C for 48 hours of incubation. To investigate the extract's anti-bacterial effects, the optical density at 620 nm of the tubes was measured.

2.4.2 Agar-Diffusion Technique

This technique allows for the study of antibacterial efficacy on an agar medium, where the bioactive chemical can exhibit zones of inhibition. For homogeneous bacterial growth as a lawn culture, the test organism was distributed plate injected over a plate of nutrient agar media. 10 µl of a chemical extract in water were used to examine the substance's antibacterial properties. After 36–48 hours of 37°C incubation, the plates were examined for the zone of inhibition surrounding the paper disc. The well's surrounding inhibitory zone has a millimeter-wide diameter (mm). The procedure described by was applied using the Agar diffusion method[21].

3.0 Results and Discussion

3.1 Spectral Analysis UV-Visible Absorption

In good agreement with the earlier results, CDs showed at 283 nm is the maximum optical absorption peak with a minor extension towards the visible range (Figure 1)[22-24]. The peak presence such as the n-π* transition of carbonyl (-C=O) and the π-π* transition of doubly linked carbon (-C=C) are responsible for the peak's existence. When illuminated by a UV lamp with a 350 nm wavelength, the liquid C-dot dispersion showed green luminescence under direct sunlight.

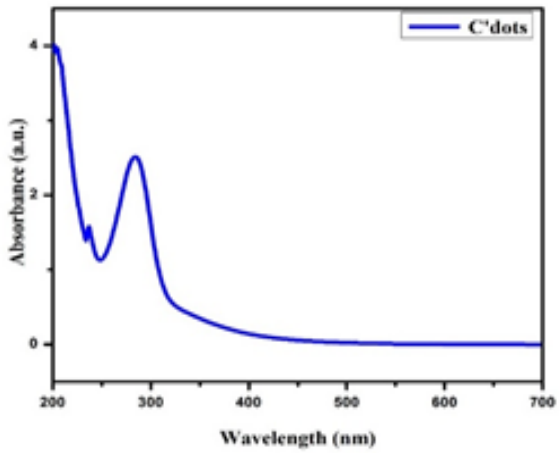


Figure 1. C-dot UV-visible absorption spectrum.

3.2 FTIR Analysis

A noticeable band around 3400-3100 cm^{-1} can be seen in the vibrational spectra of the green CDs (Figure 2), which is caused by the stretching vibration of either the -OH or -NH groups. A faint band found around 2200-2000 cm^{-1} indicates the existence of the nitrile group. A further robust absorption band between 1550–1650 cm^{-1} marks the existence of $-NH_2$ via bonds in amide, supporting the incorporation of nitrogen except for the accumulation of any source of nitrogen externally[25-27]. The indication of the out-of-plane bending of -OH bonds and C-O-C bonds respectively are demonstrated by the thin bands at 1100 cm^{-1} and 660 cm^{-1}. Therefore, not only the presence of carboxyl, hydroxyl and amino groups but also shows that there are no inorganic substances present in the system was confirmed by the FTIR spectra.

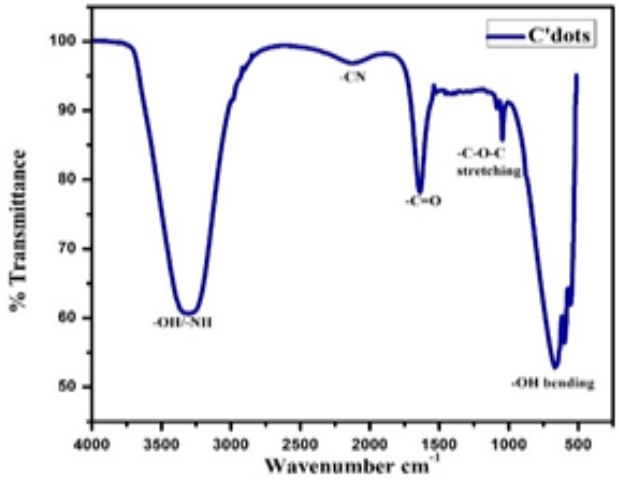


Figure 2. Carbon nanodots FTIR spectra.

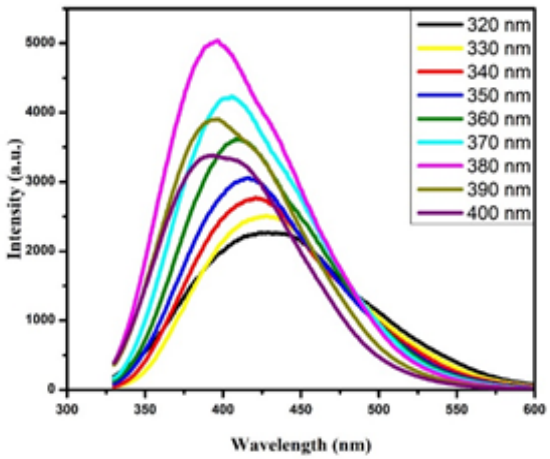


Figure 3. PL spectra of C-dots obtained at various excitation wavelengths.

3.3 Spectral Analysis Photoluminescence

To examine the optical behaviour of green produced CDs, a PL spectrum with an excitation 320 nm wavelength and an emission peak focused towards 429 nm was obtained. CDs exhibited the normal "excitation-dependent emission" behaviour, as demonstrated by several studies on fluorescence properties (Figure 3)[28-30]. The emission peak was observed to shift from 429 nm to 473 nm as the emission spectrum was increased from 320 to 400 nm, with the highest emission intensity peak being recorded at a 380 nm wave length. Such kind of validates that adjusting the excitation wavelength of synthetic CDs will cause them to emit a variety of hues, including blue, green, and red. This discovery can be explained by the optical collection of particular layer defect states close to the nanodots' Fermi level, one has previously been verified by FTIR spectra. Standard taken as 0.1 M H_2SO_4 in quinine sulphate, we were able to measure the quantum yield displayed by carbon nanodots and reached a value of up to 28.6%. This result is far greater than many of the earlier studies, particularly those from other environmentally friendly sources[31-33].

3.4 Minimum Inhibitory Concentration

The *E. coli* test culture showed a moderate increase in the OD values (Table 1) with increasing concentration of the compounds ranging from 10 µl upto 500 µl. There was no inhibition of the bacteria in the study. The gram negative

Table 1. OD value with different ranges of concentration

Concentration	*E. coli*	*Bacillus* sp.	*Pseudomons* sp.	*Klebsiella* sp.
10 µl	0.23	1.37	1.21	0.33
50 µl	0.35	1.46	1.30	0.43
100 µl	0.56	1.58	1.40	0.51
200 µl	0.58	1.54	1.0	0.56
300 µl	0.62	1.46	1.0	0.77
400 µl	0.71	1.45	1.0	0.87
500 µl	0.77	1.44	1.0	0.96

Klebsiella *sp.,* showed an increase in the optical density values with increasing concentrations of the compounds, which indicates that the compound had no bacterial inhibition. Hence it can be inferred that the compound was not effective on *Klebsiella* sp. The gram negative *Pseudomonas* sp. showed an optical density of 1.21, 1.30, 1.40 at concentrations of 10 µl, 50 µl and 100 µl. The study at 200 µl, 300 µl, 400 µl and 500 µl showed a value of 1.00 as a stabilized Optical Density value. This indicates that the compound is quite effective in inhibiting the bacterial growth. The gram-positive *Bacillus* sp. showed an optical density of 1.37, 1.46, 1.58 at concentrations of *10* µl, *50* µl and *100* µl. The study at 200 µl, 300 µl, 400 µl and 500 µl showed a value of 1.54, 1.46, 1.45 and 1.44, indicating that the compound had no effective antibacterial property[34].

3.5 Agar-Diffusion Technique

The Agar well diffusion method is one of the popular methods used to screen micro-organism for their antibacterial property. The test cultures include *Pseudomonas* sp., *Bacillus* sp., *Klebsiella* sp. was studied for antibacterial study with the test compounds using the concentrations of *50* µl *and 100* µl. The zone of inhibition studied for 50 µl showed that there were different zones of inhibition (Figure 4). The *Bacillus* sp. showed a lesser zone of 0.8 cm, whereas *Klebsiella* sp. showed 3.4 cm. The bacterium *Pseudomonas* sp. showed a better zone of inhibition with 3.9 cm and this indicates that there is an effect of the compound on these bacteria. As a conclusion the compound is effective on the gram negative bacteria, more specifically the *Pseudomonas* sp. when compared to *Klebsiella* sp. The compound showed least effect on *Bacillus* sp. and was not effective on *E. coli*.

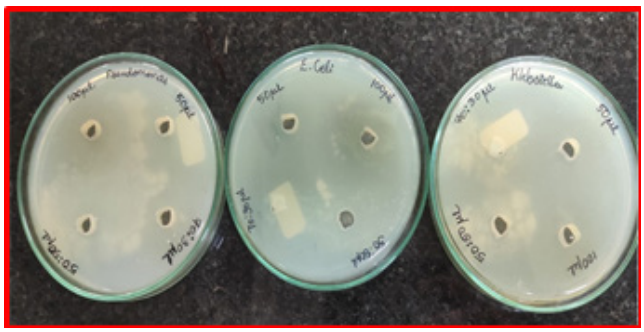


Figure 2. Zone of inhibition for 50 µl conc. against bacteria.

4.0 Conclusion

In conclusion, this study delved into the alchemical transformation of Moringa leaves into nanocarbon shards through the mineral-rich hydrothermal method. The characterisation techniques implied the development of CDs with its unique excitation-dependent wavelength emission endowed with organic functional groups such as hydroxyl, carbonyl and amino groups. By using the minimum inhibitory concentration and agar diffusion techniques, the antibacterial activity of C-dots was analysed against *Bacillus* sp., *E. coli*, *Pseudomonas* sp., and *Klebsiella*. The studies suggest that the synthesised C-dots have maximum antibacterial activity against *Pseudomonas sp.* and it reveals that Moringa leaves are an exceptional source of carbon dots with antimicrobial efficiency.

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