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# Bioactive Carbon Dots from Banana Spathe of Poovan Cultivar: A Green Approach to Nanomaterial Synthesis

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## Abstract

**Background:** Carbon dots derived from plant materials have been in the limelight in the past few years owing to their exceptional properties, conferring potential applications in biomedicine and environmental remediation. **Aim:** Properties of the green synthesised carbon dots from banana spathe, using the hydrothermal method, formed the core of this study. **Methods:** Banana spathe, a natural and safe material, was used to synthesise carbon dots, which were characterised and bioactive properties, including antioxidant, antibacterial and dye degradation. **Result:** The presence of functional groups such as hydroxyl and carboxyl groups were confirmed through UV-Vis, FTIR spectroscopy and SEM-EDX, which emphasises morphology, water solubility, stability and reactivity. The dye degradation efficiency of Banana spathe carbon dots (bsCD) was demonstrated by photocatalytic dye degradation of Congo Red (CR) with a decent decrease in absorbance in two hours. DPPH radical scavenging assay depicted good antioxidant activity of bsCD (91.1%), and well diffusion assay gave good antibacterial properties when tested on *E. coli* and *S. aureus*. **Conclusion:** The present study thus depicts the multipotent nature of carbon dots derived from a natural nontoxic material - banana spathe - marking its capabilities in the biomedical and environmental remediation domain, which are also in alignment with United Nations Sustainable Development Goals 3, 6 and 12.

**Major Findings:** Hydrothermally synthesised carbon dots from banana spathe of Poovan cultivar demonstrated strong photocatalytic degradation of Congo Red dye, increased antioxidant capability and antibacterial efficacy against Gram-negative bacteria.

**Keywords:** Antibacterial, Antioxidant, Banana Spathe, Carbon Dot, Dye Degradation

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## 1. Introduction

Carbon Dots (CDs), also known as carbon quantum dots, are sphere-shaped nanoparticles (NPs) made from carbon-based materials. CDs are photoluminescent nanoparticles and are zero-dimensional. These NCs have their surfaces attached with an enormous number of functional groups and polymer chains and a structure of sp2 and sp3 configuration[1]. Pyrolysis or hydrothermal processing of natural resources such as biomass[2], fruit juice[3], leaves[4], grass etc help in the synthesis of carbon dots at a much lower cost[5]. CDs show great significance and outstanding characteristics such as photobleaching, fluorescence property, increased electro-catalytic activity, antioxidant property, alterable emission and excitation attributes and also have great applications in forensic science, bio imaging, temperature sensors, photocatalytic applications, photothermal therapy, chemosensors, etc. These show low toxicity, water solubility, chemical inertness, great electrical conductivity and photoluminescence property, which makes these nanocarbons interesting[1,6,7].

Dyes are organic compounds that are produced on an extensive scale for industrial purposes, which give colour to various substrates, including water bodies. Depending upon the chemical structure, these dyes can

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be classified as acidic, basic, azo, cationic, anionic, etc. The discharge of these compounds increases chemical oxygen demand, creates pollution and increases the toxicity of water bodies. There is a drastic decrease in the photosynthetic process of aquatic plants due to the reduced passage of light through the affected water bodies. A few of such dyes include Congo Red, Crystal violet, Malachite green, Methylene Blue, Methyl orange, and Rhodamine-B. Absorption, flocculation, coagulation, nanofiltration, and chlorination are particular procedures that have been used for processing and treatment of contaminated water, degradation of dye and maintaining water balance[8,9]. Congo Red (CR) is an azo dye among the widely used ones in industry. CR, as it has two azo bond chromophores, is considered one of the dyes that come under the azo dye category. These azo dyes have a severe toxicological impact as well as are carcinogenic in nature, even if these dyes have great application in textile, paper, and cosmetic industries[10,11]. When dyes degrade over time, they produce intermediate products, some of which are more noxious than the dye, as in the case of benzidine, which is a breakdown product of azo dyes like CR[12]. To circumvent these problems, an effective degradation of CR is required, which could be tried with Carbon dots[8].

Substances that retard oxidative activity by obstructing reactive species and derivatives are known as antioxidants. These can be either enzymatic or non-enzymatic. Factors influencing their bioavailability include instability, inadequate radical scavenging, and poor absorption ability for both synthetic and natural antioxidants. In biological processes, there is a production of radicals and reactive oxygen species, which, in greater amounts, lead to oxidative stress. Carbon dots, which are synthesised either doped or undoped, show strong antioxidant properties which protect the cells from oxidative stress[13,14]. Flavonoids and phenols are compounds that contribute to the antioxidant properties of the banana spathe. The damage to cells caused by free radicals is protected by these compounds, thereby preventing disease and enhancing health. DPPH-based antioxidant properties are studied in this paper.

CDs, being pollution-free and nontoxic in nature, exhibit antimicrobial or antibacterial properties. These Cardon dots, therefore, can be utilised on behalf of commercial antibacterial chemicals, which are lethal to environmental systems. Antimicrobial activity can be tested using a zone of inhibition test, wherein the size of the zone indicates the antibacterial or antimicrobial activity[15]. The bract that protects the banana flower, known as the banana spathe, is a highly protective outer covering. It easily attracts the pollinators, drawing them for pollen, due to its colour and shape. These outer coverings have great antioxidant properties as well as a complex inflorescence structure. Banana spathe is also a rich reservoir of phytochemicals[8]. In this study, banana spathe, a natural and safe material, was used to synthesise carbon dots, which were characterised, and bioactive properties, including antioxidant, antibacterial and dye degradation, were explored.

## 2. Materials and Methods

### 2.1 Preparation of Banana Spathe Powder

Banana spathe was collected from the Poovan cultivar of *Musa acuminata* from Kannur, Kerala. The material was sliced into smaller pieces and sundried for 3 days. The material was powdered in a mixer grinder and was used for synthesising the carbon dots.

### 2.2 Synthesis of bsCDs

Banana spathe Carbon Dots (bsCDs) were synthesised using the hydrothermal method using a Teflon autoclave. 3g of the banana spathe powder was taken in a beaker, and 30 ml of deionised water was poured into it. The solution was stirred thoroughly and transferred into a Teflon autoclave and was heated at 180°C for 6 hours. After cooling, filtration of the solution was done using Whatman filter paper (0.22 µm) and centrifuged at 8000 rpm for 15 min. The supernatant was then syringe filtered and stored. This CD solution was then pyrolysed at 300°C for 2 hours and was stored for performing antioxidant, antibacterial and dye degradation tests[7].

### 2.3 Characterisation

The synthesised biomass-derived CDs were characterised using UV-Visible spectroscopy (UV-Vis) and Fourier Transform Infrared Spectroscopy (FTIR). The morphology was then studied using scanning electron microscopy coupled with Energy-dispersive X-ray spectroscopy (SEM-EDS).

## 2.4 Dye Degradation

A 10 ppm stock solution (100ml) of CR dye was prepared using distilled water. 0.5ml of CD solution (10 mg/ml) was mixed with 5ml CR dye solution in the test tube. Similarly, 0.5 ml CD solution was mixed with 5 ml distilled water in another test tube to serve as a control. The solutions were then exposed to light for illumination. Absorbance was measured using a UV-Vis spectrophotometer at 497 nm at different intervals of 30, 60, 90 and 120 min. The degradation percentage of dye was also calculated using the formula given below[11].

Degradation percentage (%) = $[(A_0 - A_t) / A_t] \times 100$

Where $A_0$ is the initial absorbance of the dye solution, and $A_t$ is the absorbance at time t.

## 2.5 Antioxidant Activity by DPPH Assay

DPPH radical scavenging activity was used for analysing the antioxidant activity of bsCDs. The CD solution was mixed with 0.1mM DPPH solution and methanol. This was then incubated at room temperature for 30 min under dark conditions. Absorbance values were recorded at 520 nm with Ascorbic acid as the standard antioxidant. The radical scavenging activity was then calculated using the following formula[16,17].

Radical scavenging activity (%) = [(Absorbance control – Absorbance sample) /Absorbance control] × 100

## 2.6 Antibacterial Activity Test by the Well Diffusion Method

Mueller-Hinton agar media was employed for the well diffusion method to test antibacterial activity. 16-hour-old cultures of the Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* were spread-plated. A clean, sterile cork borer was utilised to create a well of approximately 9mm diameter. The wells were loaded with negative control (deionised water), positive control (Ampicillin) and the CD sample. The plates were then incubated for 24 hours at 37°C, after which the inhibition zones were measured and recorded in mm[18,19].

# 3. Results and Discussion

## 3.1 Synthesis and Characterisation of bsCDs

### *3.1.1 UV-Vis Spectroscopy*

CDs from the spathe of the Poovan cultivar of banana were synthesised by the hydrothermal method (Figure 1). Crucial insights into the optical properties of green-synthesised carbon dots were revealed through UV-Vis spectroscopy (Figure 2a). The spectrum displays strong absorption within 240-260 nm, attributed to π-π* transitions of $sp^2$ hybridised carbon dots, with high absorbance indicating efficient light absorption characteristic of carbon dots. A gradual decline was observed at higher wavelengths. This profile consists of n-π* transitions in nitrogen-containing groups as well as in carbonyl groups (C=O) and π-π* transitions in aromatic C=C bonds, and similar absorbance was reported due to graphitic and surface oxygen or nitrogen-rich structure of folic acid-derived CDs[20]. The n-π* and π-π* transitions are indicative of conjugated carbon core and heteroatom-rich surface functional groups, which are mainly observed in green-synthesised carbon dots. This absorption profile is typical in CDs wherein the abundance of carbon and oxygen mass results in blue or green fluorescence[21].

### *3.1.2 FTIR Analysis*

The successful synthesis and functionalization of green-synthesised carbon dots were confirmed through the FTIR spectrum (Figure 2b). The O-H stretching vibrations around 3400-3300 $cm^{-1}$ indicate the presence of hydroxyl groups on the CD surface. These peaks contribute to the hydrophilicity and water solubility of carbon dots. The peaks around 2920-2850 $cm^{-1}$, 1700-1600 $cm^{-1}$ and 1400-1300 $cm^{-1}$ are attributed to C-H stretching, C=O stretching, and C-H bending vibrations, respectively. This indicates the presence of aliphatic C-H bonds, chemical reactivity and stability. The 1200-1000 $cm^{-1}$ peaks are indicative of C-O stretching vibrations[20]. These surface functionalities are typical in green-synthesised carbon dots and are used in metal ion sensing or drug delivery, and even essential for biocompatibility[21]. The present data suggest that the bioactivity and antioxidant properties of CDs are contributed by the polar functional groups such as –OH, -COOH and $-NH_2$[22]. The sample of bsCDs was also showing fluorescence under UV light (Figure 2c).

### *3.1.3 SEM-EDX Analysis*

The SEM image, as depicted in Figure 2d, reveals that bs-CDs show a shape consistent with green synthesised carbon nanoparticles produced from biomass. The observed particles are mostly quasi-spherical




**Figure 1.** Green synthesis of Carbon dots from banana spathe; **(a).** Banana, *Musa acuminata* cultivar Poovan (AAB); **(b).** Banana spathe pieces; **(c).** Sun-dried banana spathe; **(d).** Powdered banana spathe; **(e).** Teflon autoclave used for carbon dot synthesis; **(f).** Carbon dots synthesised from banana spathe.

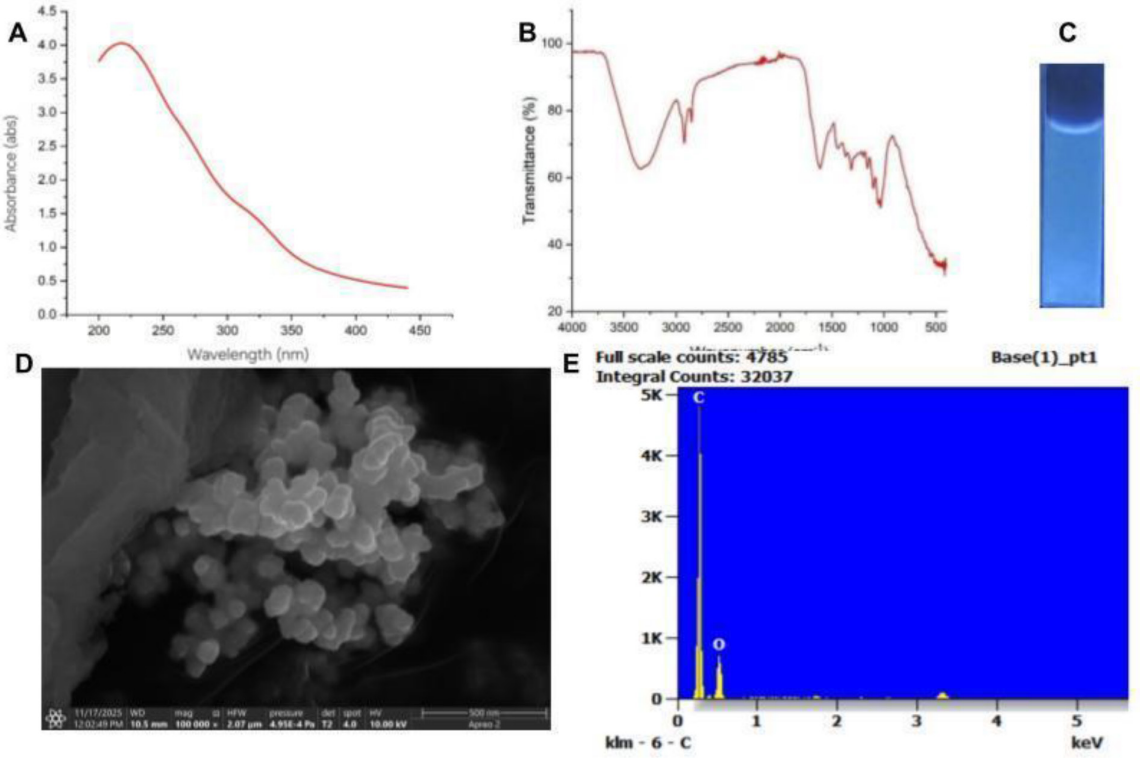



**Figure 2.** Characterisations for biosynthesised Carbon dots from banana spathe; **(A).** UV-Vis spectrum of banana spathe carbon dots; **(B).** FTIR spectrum for banana spathe carbon dots; **(C).** Fluorescence under UV light; **(D).** Energy-dispersive X-ray spectroscopy spectrum, showing prominent peaks for carbon and oxygen; **(E).** SEM analysis for banana spathe carbon dots (100,000X magnification).

and appear well dispersed, though there is some aggregation, which is commonly attributed to drying and sample preparation steps. This aggregation is commonly observed for biosynthesised carbon dots as biomass degradation produces carbon skeletons attributed to heteroatoms, residual surface functional groups and incomplete isolation during synthesis, as shown for other plant-derived carbon dots, including banana peel, banana pseudostem-derived carbon dots and coconut shell-sourced carbon dots[7,23,24].

The EDX results (Figure 2e) show the elemental composition of banana spathe-derived carbon dots, with carbon (C) at 65.15 atom % and oxygen (O) at 34.85 atom %, indicating a high carbon-rich phase with significant oxygen functionalisation, as is typical of biomass-derived carbon nanomaterials. Carbon dominates in the net counts and weight/atom percentages, which are comparable with hydrothermal carbonisation results from banana biomass. The significant oxygen fraction is a hallmark of surface functionalization, which enables solubility and fluorescence for carbon dots. Studies show that banana biomass conversion results in carbon percentage between 61-72 atom% and oxygen remains between 26-39 atom%, which is similar to this study. These ratios result from partial oxidation and dehydration during hydrothermal or pyrolytic treatments, and they represent the presence of hydroxyl, carboxyl and ether linkages, which are required for optical characteristics and use in sensing and catalysis[25,26].

## 3.2 Degradation of Congo Red Dye using bsCDs

CR was used for estimating the dye degradation properties of bsCDs. This azo dye has been found to cause extensive water pollution[27,28] and is also found to be detrimental to various organ systems of aquatic animals[29], as it is still found as a commercial effluent which are dumped into water bodies in many countries. A 10 ppm solution of Congo Red, when incubated with bsCDs, showed a good degradation of about 40% even in a short time of 2 hours (Figure 3). The positive result for the reduction in absorbance implies the significant dye degradation property of CDs.

Generally, those carbon dots which are synthesised through green methods showcase photocatalytic degradation of dyes[11]. Numerous studies explain the properties of CDs that attach the dye molecule, such as optical activity, high surface area and great reactive oxygen species generation. CDs without toxic dopants showed degradation of dyes such as Congo Red, as well as Methylene blue[30]. The increased degradation of dye is credited to the breakdown of dye molecules upon exposure to sunlight, which reacts with oxygen molecules. The present study also portrays a similar mechanism, highlighting the potential of CDs as sustainable agents for the treatment of wastewater and proves the photo-catalytic degradation of dye using green-synthesised carbon dots, thereby making it environmentally friendly.

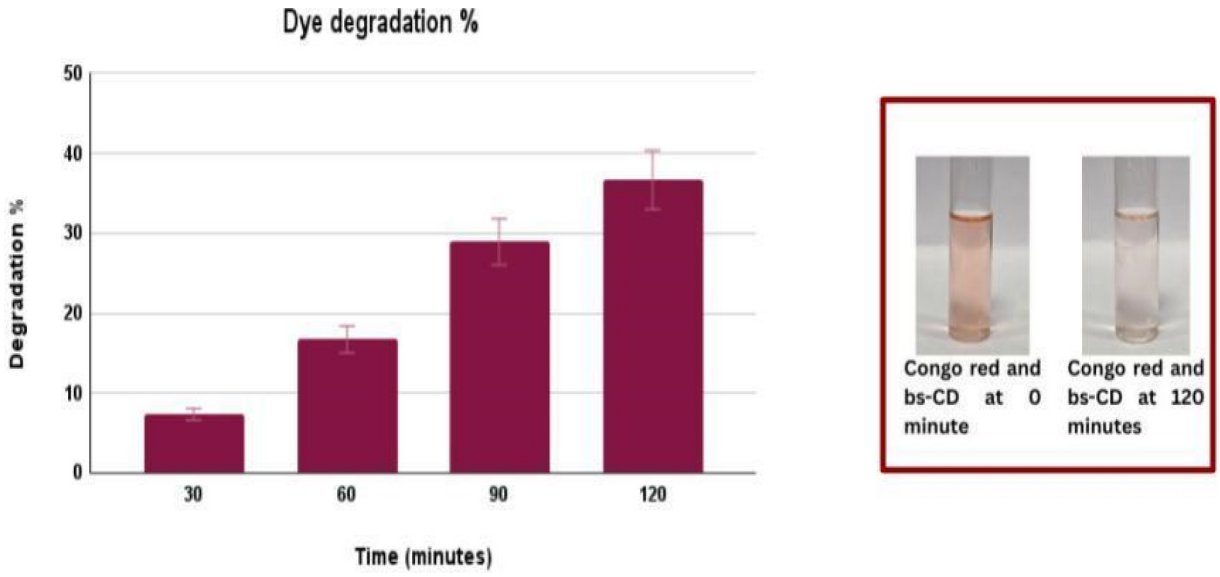



**Figure 3.** Degradation of Congo Red dye under the influence of green-synthesised carbon dots from banana spathe.

### 3.3 Antioxidant Activity of bsCDs

DPPH radical scavenging was used to study the antioxidant potential of bsCD. A clear increase based on the concentration was observed in the Radical Scavenging Activity (RSA%). bsCDs showed an average RSA% of approximately 67.5% at lower concentration (2.5 mg/ml) and increased to 91.1% at higher concentration (10 mg/ml) as depicted in Figure 4. The standard antioxidant, Ascorbic acid, showed a very good result of 98.6% RSA. The change in colour from violet to yellowish brown confirms DPPH radical reduction, which is an indicator of effective antioxidant activity[31].

Our results are consistent with various studies. A significant antioxidant activity has been showcased by carbon dots synthesised from medicinal plants due to the existence of hydroxyl and carboxyl functional groups[16]. The bsCDs exhibit similar chemistry, allowing them to neutralise free radicals. The capacity of banana spathe carbon dots to behave as redox modulators, taking part in electron transfer not just with DPPH radicals but also with other ROS and reactive nitrogen species in a biological setting, is a key and important mechanism supporting their antioxidant effect. This mechanism is also supported in a 2020 study by Murru *et al.*, wherein carbon dots were shown to scavenge reactive oxygen in both aqueous and oil environments. The scavenging activity of carbon dots is due to the redox-active nature of carbon dots, wherein CDs scavenged oxygen and nitrogen species[32]. Although not as efficient as ascorbic acid, the bsCDs generated by the present study also demonstrated strong antioxidant activity, making it a useful agent in biomedical and cosmetic formulations as eco-friendly antioxidants.

### 3.4 Antibacterial Activity of bsCDs

CDs are expected to possess good antimicrobial activity, essentially owing to their nanostructures. Green synthesised bsCDs were evaluated for their antibacterial property using the agar well diffusion method, wherein *E. coli* (gram-negative) and *S. aureus* (gram-positive) were used as bacterial strains. Bacteria of different types were chosen to check if both types are equally vulnerable to the damaging action of CDs. In the well diffusion assay, good inhibitory potential was depicted in the case of Gram-negative bacteria (Figure 5), which displayed zones of inhibition measuring 12mm at 1mg/ml and 16mm at 2mg/ml concentrations. The inhibition was lacking for Gram-positive bacteria. Ampicillin, the standard antibiotic, resulted in 19mm inhibition for *E. coli* and 18mm for *S. aureus*. This differential inhibitory activity can be correlated with the inherent differences in the cell wall structure of Gram-positive and Gram-negative bacteria, with the latter having more lipid-rich materials in the cell wall.

An abundance of functional groups, such as hydroxyl and carboxyl groups, was found in the carbon dots, which facilitate drastic interactions with the cell walls of bacteria[18]. Effective bacterial cell death,

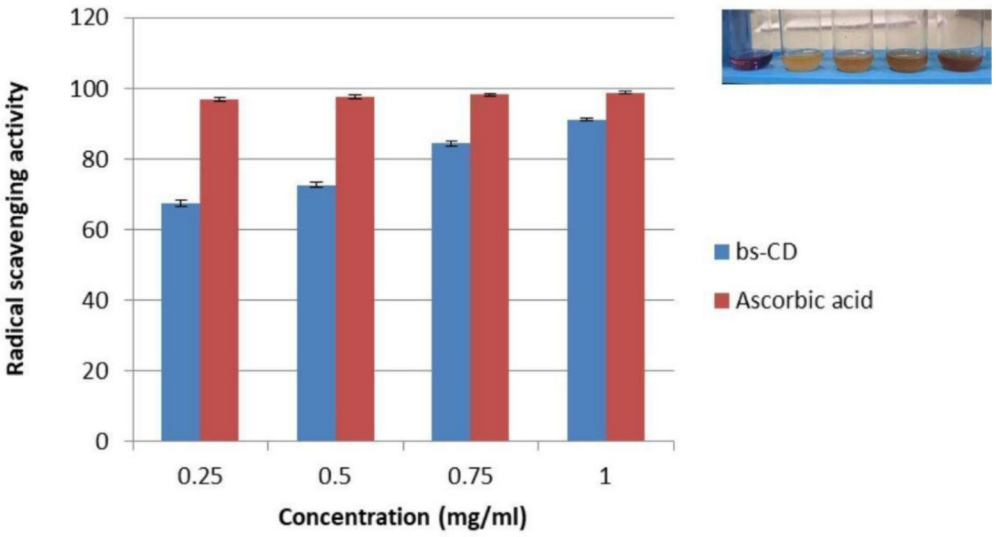


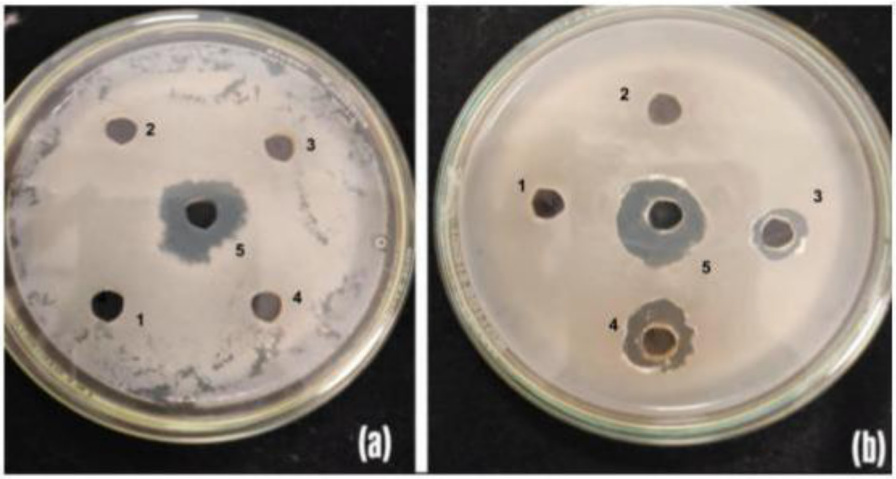

**Figure 4.** DPPH assay measures the radical scavenging activity of banana spathe carbon dots and Ascorbic acid.

especially in gram-negative strains, could be observed due to oxidative stress through reactive oxygen species in those carbon dots synthesised from medicinal plants[16]. Gram-negative strains of bacteria with a thin peptidoglycan layer and outer membrane are more vulnerable to destruction by nanoparticles compared to gram-positive ones having more resistance[6]. The activity of green synthesised CDs effectively inhibiting *E. coli* highlights its potential as an eco-friendly agent, whereas the absence of similar activity in the *S. aureus* strain suggests that certain green synthesised CDs have a limited spectrum, also suggesting their role in bacterial growth inhibition via cell wall peptidoglycan damage.

## 3.5 Applications of bs-CDs

Diverse plant sources are used to synthesise carbon dots through various methods such as hydrothermal, solvothermal and microwave methods, as depicted in Table 1. These green synthesised carbon dots exhibit a variety of applications across forensic, biomedical and environmental domains. CDs from *Cupressus lusitanica*, curry leaves, rosemary leaves, Kaempferol, and many plants used in Chinese herbal medicine possess forensic applications. Banana pseudo-stem and coriander leaves demonstrate bioimaging and sensor capabilities. A more detailed analysis of these CDs could be done using XPS, TEM and even Raman spectroscopy, which offers drastic insight into their morphological and surface chemistry. These applications emphasise CDs as a sustainable alternative for real-world nanotechnological applications.

Banana spathe has a rich repertoire of bioactive compounds which are responsible for its antioxidant, antibacterial and anti-inflammatory properties. Rutinosides are one of the major anthocyanin groups of compounds found in the banana spathe, offering such attributes. A few of their structures are depicted in Figure 6, and their bioactive properties are listed in Table 2. The green synthesised carbon dots show tremendous potential in accomplishing goals 3 and 6 of Sustainable Development of the United Nations. The strong antioxidant and antibacterial properties of Carbon dots hint at their potential utility in human




(c)

| | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| Bacterial species | Negative control (1) | Carbon dots 0.5 mg/ml (2) | Carbon dots 1mg/ml (3) | Carbon dots 2 mg/ml (4) | Ampicillin (5) |
| *S. aureus* | - | - | - | - | 18 |
| *E. coli* | - | - | 12 | 16 | 19 |

**Figure 5.** Antibacterial activity of banana spathe carbon dots (bsCDs) with; **(a).** *S. aureus* (Gram positive) bacteria and; **(b).** *E. coli* (Gram negative) bacteria; **(c).** Zones of inhibition - 1: Negative control (deionized water), 2: 0.5 mg/ml carbon dots, 3: 1 mg/ml carbon dots, 4: 2 mg/ml carbon dots, 5: Standard antibiotic Ampicillin as positive control.

**Table 1.** Applications of green-synthesised carbon dots derived from various plant sources

| Carbon Dot Source Plant | Synthesis Method | Application | References |
|---|---|---|---|
| *Cupressus lusitanica* | Microwave method | Fluorescent ink, development of latent fingerprint, antibacterial, WLEDs | 19 |
| Kaempferide plant | Hydrothermal method | Anti-counterfeiting ink | 35 |
| Chinese herbal medicine | Solvothermal method | Anti-counterfeiting, $Fe^{3+}$ sensing, photodegradation | 36 |
| *Magnolia grandiflora* | Hydrothermal method | Latent fingerprint detection | 37 |
| *Murraya koenigii* | Microwave method | WLEDs, Anti-counterfeiting ink | 38 |
| *Salvia rosmarinus* | Hydrothermal method | Antimicrobial activity, food storage capacity, fingerprint detection | 6 |
| Pseudostem of the banana plant | Hydrothermal method | Nano sensor, bio imaging | 7 |
| *Coriandrum sativum* | Hydrothermal treatment | Antioxidants, sensors, bioimaging | 4 |
| *Chionanthus retusus* | Hydrothermal carbonisation | Metal ion sensing, fluorescence imaging | 39 |

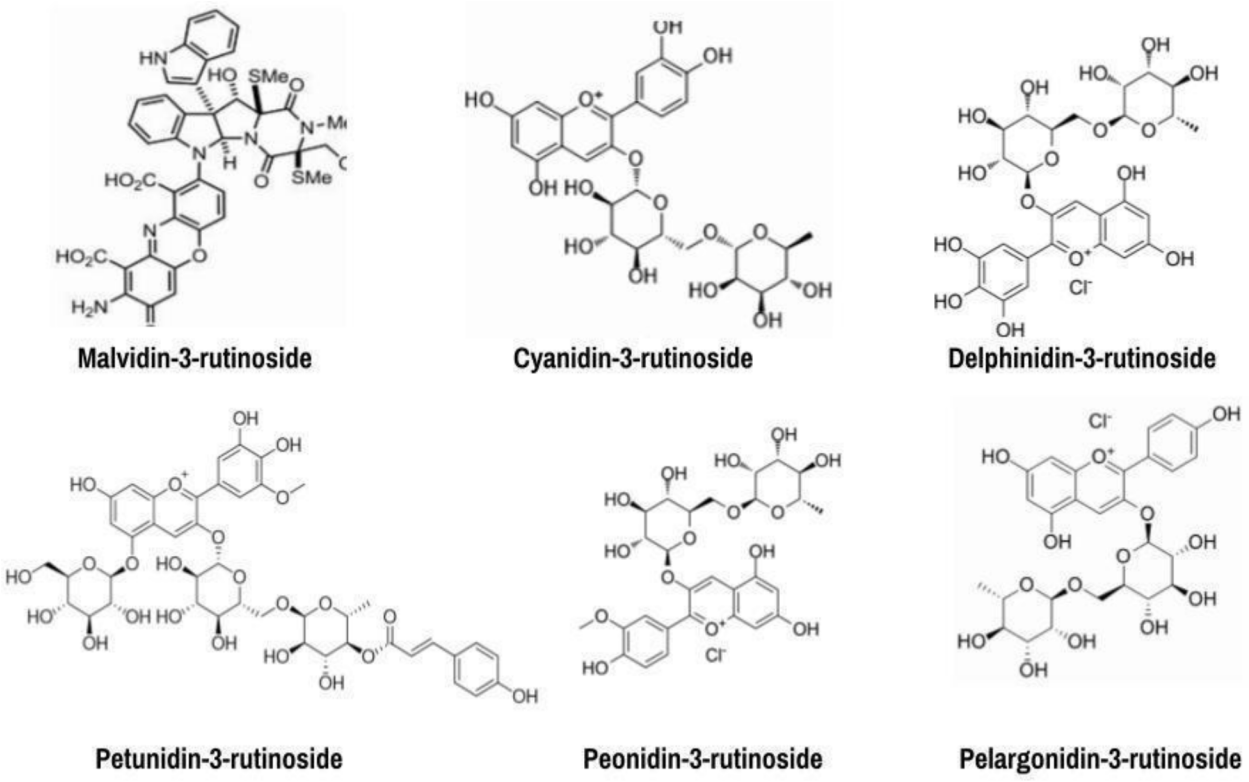



**Figure 6.** Structures of prominent rutinosides with proven bioactivities in the spathe of the Poovan variety of banana.

health and wellbeing, and also align well with SDG3. The ability of carbon dots in breaking down dangerous dyes in water ensures the accessibility of clean water, which aligns with SDG6[33,34].

## 4. Conclusion

Green synthesis of carbon dots using banana spathe as a carbon source was successfully done in this study using hydrothermal synthesis methods. The

**Table 2.** Bioactive properties of prominent rutinosides of the Poovan variety of banana

| Compound | Bioactivity | References |
|---|---|---|
| Cyanidin-3-rutinoside | Inhibition of the metastasis of human lung carcinoma cells | 40 |
| Delphinidin-3-rutinoside | Inhibition of 3 diabetes-related enzymes: Dipeptidyl peptidase-4 (DPP-4), Alpha-amylase, and Protein tyrosine phosphatase 1B (PTP1B) | 41 |
| Delphinidin-3-rutinoside | Antiproliferative effect in gastric and colon cancer cell lines | 42 |
| Petunidin-3-rutinoside | Strong antioxidant activity along with cytoprotective effects. Provides insights into flavonoid metabolism | 43 |
| Peonidin-3-rutinoside | Inhibition of cancer metastasis through modulation of MAPK | 44 |
| Malvidin-3-rutinoside | Inhibition of cell proliferation, induction of apoptosis and cell arrest. Suppression of invasion via MAPK and PI3K/AKT pathways | 45 |
| Pelargonidin-3-rutinoside | Inhibits inflammation by reducing mediators like NO, TNF-α and IL-6 and reduces cardiovascular risk factors. | 46 |

presence of vital functional groups that contribute to photocatalytic, antioxidant and antibacterial properties was substantiated using characterisation. A concentration-specific antioxidant activity was demonstrated by DPPH radical scavenging assay reaching upto 91.1%, exhibiting excellent antioxidant potential of bsCDs. The antibacterial property showed a good zone of inhibition against *E. coli* strains. The photocatalytic degradation of Congo red upon exposure to sunlight supports the utility of bsCDs as agents for wastewater treatment. The promising results highlight the potential of bsCDs as an agent used for biomedical and environmental remediation and banana spathe as a low-cost, natural and safe biomass precursor for synthesising efficient CDs. The paper provides an insight into future advancements in nanotechnology using agricultural waste/byproducts as safer bioactive materials.

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## Author Contribution

MPV: Methodology, Investigation, Original draft; SS: Conceptualization, Supervision and Editing. All authors have read and agreed to the published version of the manuscript.