

# **Hydrothermal Synthesis of Carbon Dots from *Lawsonia inermis* Plant Extracts and Their Antimicrobial Activities**



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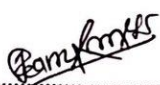
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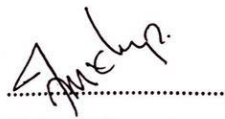
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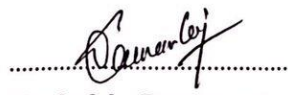
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**Susmita Panthi Upadhyay**

## ABSTRACT

*Lawsonia inermis* is recognized for its medicinal and dyeing applications for exploring its potential in the domain of nanotechnology, specifically, in the synthesis of Carbon Dots (CDs). This study uses the hydrothermal synthesis of CDs utilizing *Lawsonia inermis* leaves extract, underscoring an eco-friendly methodology and harnesses the plant's phytochemical richness but also aligns with green chemistry principles. Carbon dots, a subset of quantum dots characterized by their zero-dimensional structure and size of less than 10 nm, exhibit fluorescence due to quantum confinement effects, surface states, and other unique properties. Their smaller size and reduced toxicity make them ideal candidates for various applications, including infectious disease treatment, biosensing, bioimaging, gene delivery, catalysis, and targeted drug delivery systems.

In this work, the hydrothermal approach facilitated the green synthesis of CDs, which were subsequently observed to emit green fluorescence under a UV lamp at 365 nm. The characterization of these CDs was carried out using UV-visible spectroscopy, FTIR, and XRD techniques, which collectively confirmed the successful synthesis and provided insights into their structural and chemical attributes. Moreover, the zeta potential measurement underscored the colloidal stability of the CDs, with a value determined to be -29 mV. The antimicrobial efficacy of the synthesized CDs was evaluated through the agar well diffusion method against prominent bacterial strains, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and one fungal *C. albicans*. The results demonstrated significant zones of inhibition, indicating the CDs' potent antibacterial activity and suggesting their applicability as a potential antimicrobial agent.

*Keywords: Lawsonia inermis, leaves extract, Carbon dots, Hydrothermal synthesis,, Antimicrobial activity*

## शोध सार

मेहेन्दी (*Lawsonia inermis*), सामान्यता औषधीय र रंगाई प्रयोगको लागि परिचित बिरुवा हो । न्यानोटेक्नोलोजिको क्षेत्रमा यसलाई विशेष गरी, कार्बन डट्स (CDs) निर्माणको लागि प्रयोग गर्न सकिन्छ । CDs, क्वान्टम डट्सको उपसमूह अन्तर्गत पर्ने १० नानोमीटर भन्दा कमको आकार भएको एक प्रकारको शून्य-आयामी संरचना हो जसले क्वान्टम प्रभावहरू, सतहको अवस्थाहरू, र अन्य विशेष गुणहरूको कारणले फ्लोरोसेन्स प्रदर्शन गर्दछ । तिनीहरूको सानो आकार र कम विषाक्तताले तिनीहरूलाई संक्रामक रोग उपचार, बायोसेन्सिङ, बायोइमेजिङ, जीन डेलिभरी, क्याटालिसिस, र लक्षित औषधि वितरण प्रणाली जस्ता विभिन्न अनुप्रयोगका लागि उपयुक्त बनाउँछ ।

यस अध्ययनमा मेहेन्दी पातको एक्स्ट्याक्ट प्रयोग गरेर हाइड्रोथर्मल विधिद्वारा CD हरूको स्यन्थेसिस गरिएको थियो । यस अनुसन्धानमा, हाइड्रोथर्मल विधिले CDs को ग्रीन स्यन्थेसिसलाई सहज बनाएको छ, जसले ३६५ नानोमीटर को यु भी प्रकाशमा हल्का हरियो फ्लोरोसेन्स प्रदर्शन गर्दछ । तिनीहरूको विशेषता मुल्यांकन यु भी स्पेक्ट्रोस्कोपी, FTIR र XRD प्रविधिहरू प्रयोग गरी गरिएको थियो, जसले सामूहिक रूपमा सफल स्यन्थेसिस भएको पुष्टि गर्‍यो र तिनीहरूको संरचनात्मक र रासायनिक विशेषताहरूमा अन्तर्दृष्टि प्रदान गर्‍यो। जेटा पोटेन्सीयल मापनले (२९ मिलिभोल्ट) कोलोइडल स्थिरतालाई पुष्टि गर्‍यो । स्यन्थेसिस CDs हरूको एन्टिमाइक्रोबियल प्रभावकारिता *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* र एउटा फंगल *Candida albicans* स्ट्रेनहरू विरुद्ध अगार वेल विधिद्वारा मूल्याङ्कन गरिएको थियो । यसको परिणामले विभिन्न अवरोधका क्षेत्रहरू देखाएर CDs को एन्टिमाइक्रोबियल गुणलाई पुष्टि गर्छ र तिनीहरूलाई सम्भावित एन्टिमाइक्रोबियल एजेन्ट को रूपमा प्रयोग गर्न सुझाव दिन्छ ।

**शब्द कुञ्जिका :** मेहेन्दी, कार्बन डट्स, हाइड्रोथर्मल स्यन्थेसिस, एन्टिमाइक्रोबियल

## LIST OF ACRONYMS AND ABBREVIATIONS

CDs	Carbon Dots
µg/mL	Micrograms per Milliliter
ATCC	American Type Culture Collection
ATR	Attenuated Total Reflectance
DNA	Deoxyribonucleic Acid
FTIR	Fourier Transform Infrared Spectroscopy
g	Gram
H1	Hydrothermal sample
IoST	Institute of Science and Technology
MBC	Minimum Bactericidal Concentration
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
mL	Milliliter
mm	Millimeter
mV	Millivolt
MV	Megavolt
nm	Nanometer
pH	Potential of Hydrogen ( <i>puiissance de hydrogen</i> )
Psi	Pound force per square inch
QDs	Quantum Dots
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
rpm	Revolution per minute
SEM	Scanning Electron Microscopy
T.U.	Tribhuvan University
TEM	Transmission Electron Microscopy
UV	Ultraviolet
UV-vis	Ultraviolet-Visible Spectroscopy
XRD	X-Ray Diffraction

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# CHAPTER 1

## INTRODUCTION

### 1.1 Background

Nanotechnology is a rapidly growing field with numerous industrial applications: notably in healthcare for drug delivery, diagnostic tools, and imaging technologies, and in enhancements related to manufacturing, product lifespan, and bioavailability (Malik et al., 2023; Patra et al., 2018). Nanomaterials have distinctive properties including catalytic efficiency, conductive capabilities, and enhanced biological and optical characteristics, owing to their increased surface area and specific size and shape attributes compared to their bulk counterparts (Altammar, 2023; Baig et al., 2021). These unique qualities have led to their application in diverse areas such as antimicrobial treatments, electronic devices, catalysis, medication delivery, water purification, and photocatalytic processes (Garg et al., 2022; Harish et al., 2022).

Nanomaterials come in various morphologies, including nanoparticles, nanorods, nanowires, nanotubes, nanobelts, nanofibers, and quantum dots. Carbon-based structures like fullerenes and polymers, as well as metallic (e.g., Au, Ag, Cu), semi-metallic, magnetic, and ceramic materials, represent the broad spectrum of nanomaterial classifications (Sudha et al., 2018). They are further categorized by their dimensional properties into zero-dimensional, one-dimensional, two-dimensional, and three-dimensional materials (Tiwari et al., 2012), with zero-dimensional nanomaterials including quantum dots and hollow spheres, all of which are entirely within the nanoscale (Wang et al., 2020).

Synthesis of nanomaterials can be achieved through physical, chemical, and biological methods, with the latter involving the use of microorganisms, enzymes, fungi, plants, or plant extracts (Alsaiani et al., 2023; Mustapha et al., 2022). Hydrothermal synthesis approach is favored for its environmental friendliness and reduced toxicity, offering a sustainable alternative through the use of plant extracts as natural reducing and stabilizing agents (Mustapha et al., 2022). Hydrothermal synthesis is recognized for its simplicity, cost-effectiveness, and lower risk of toxic byproducts, making it a highly efficient method for nanomaterial production.

The advent of CDs represents a significant breakthrough in nanotechnology. Characterized by their particle size of less than 10 nm, CDs exhibit exceptional properties such as high fluorescence, chemical stability, and biocompatibility, which

make them ideal for various applications including bioimaging, sensing, and photocatalysis (Anwar et al., 2019; Behi et al., 2022; Jorns & Pappas, 2021). The unique optical properties of CDs, such as their tunable emission and excitation wavelengths, enhance their utility in medical diagnostics and environmental monitoring, underscoring the versatility of these nanomaterials (Anwar et al., 2019).

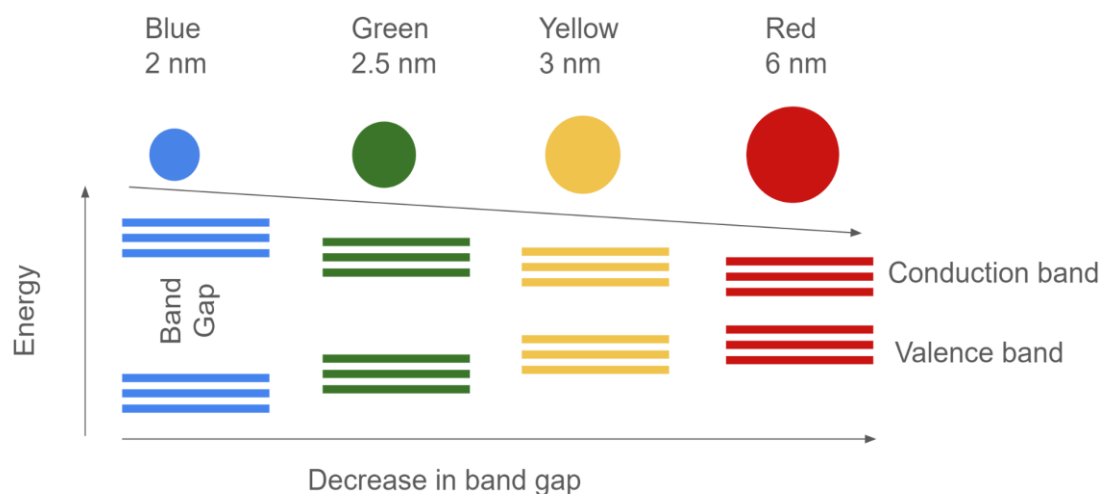
Moreover, the synthesis of CDs using green chemistry principles aligns well with current sustainability goals. The use of natural precursors, particularly plant extracts, for the synthesis of CDs not only minimizes the environmental impact but also enhances the eco-friendliness of the production process. Plant-derived CDs are noted for their ease of functionalization, which allows for the incorporation of various bioactive compounds that can target specific biomedical or environmental applications (Xiang & Tan, 2022). This bio-based approach not only leverages the intrinsic properties of plants but also exploits their natural metabolic pathways to yield CDs with enhanced performance. As research progresses, the potential of CDs to be synthesized from a variety of botanical sources suggests a promising direction for future innovations in both material science and applied nanotechnology. These developments are likely to expand the applicability of CDs beyond traditional bounds, opening up new frontiers in both industrial and clinical settings.

## **1.2 Quantum Dots**

Quantum dots (QDs) are semiconducting crystals with dimensions of 2 to 10 nm, made of group II-VI or III-V elements (Mohamed et al., 2021). These nanocrystals consist of a semiconductor core enveloped by a shell and capped with agents that enhance their solubility in water (Mohamed et al., 2021; Reiss et al., 2009). QDs exhibit unique optical properties including tunable light emission based on size and composition, broad absorption spectra, and high fluorescence intensity with significant quantum yields. The ability of QDs to absorb white light and re-emit it in specific colors shortly after is a direct consequence of their material band gap, with smaller dots shifting the emission spectrum towards the blue end is termed as quantum confinement (Brazis, 2017).

Quantum dots can be synthesized through both top-down approaches, like lithography and etching, and bottom-up techniques, including chemical synthesis and bio-based methods. Their unique chemical and physical characteristics allow for their use in a wide array of products and technologies, ranging from solar panels and electronic

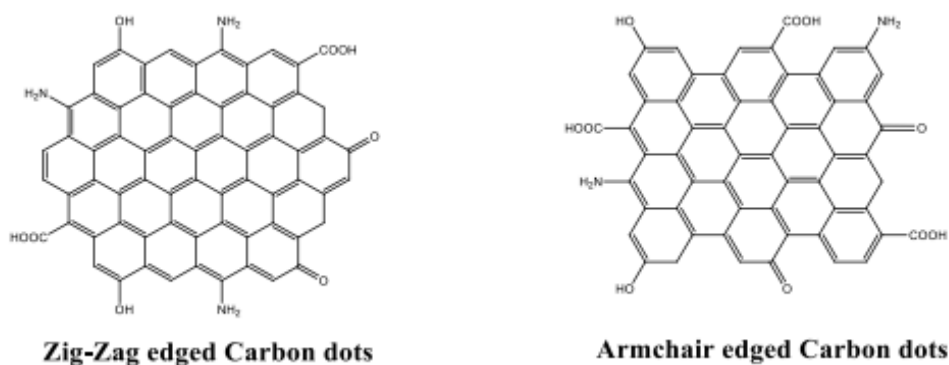
devices to biomedical applications and environmental purification systems (Mohamed et al., 2021).



**Figure 1:** Schematic diagram to show the correlation between size of CDs particle and band gap..

### 1.3 Carbon Dots

Carbon dots (CDs) fall within the category of quantum dots, representing a new class of carbon-based, zero-dimensional nanoparticles with sizes under 10 nm. The quasi-spherical particles, also known as carbon nanodots or fluorescent carbon quantum dots, are distinguished by their strong UV light absorption, high fluorescence emission, excellent water solubility, biocompatibility, and environmentally friendly nature (Jorns & Pappas, 2021). The structural composition of CDs varies, with crystalline or amorphous cores surrounded by hydrogen or functional groups like  $\text{NH}_2$ ,  $\text{OH}$ ,  $\text{COOH}$ , emphasizing their adaptable nature for various applications (Huo et al., 2020).



**Figure 2:** Zig-zag and Armchair pattern of CDs

The structural versatility of carbon dots allows for a variety of synthesis techniques and functional outcomes. Typically, CDs can have either crystalline or amorphous cores which are densely packed with carbon atoms. The surfaces of these cores are richly

functionalized with various organic groups such as amino  $\text{NH}_2$ , hydroxyl (OH), and carboxyl (COOH). These surface modifications contribute to the water solubility and compatibility of CDs with biological environments and enable precise interactions with specific molecules, thereby expanding their utility in targeted drug delivery and diagnostic applications.

Figure 2 illustrates the typical zig-zag and armchair patterns found in the lattice structures of carbon dots. These structural variations influence the electronic properties of CDs, particularly their conductive and fluorescent behaviors. The zig-zag edges in a carbon dot tend to exhibit metallic-like conductivity, whereas armchair edges are more semiconductor-like. Understanding these patterns is important for tailoring CDs for specific electronic and optical applications, as they directly affect the quantum confinement and edge effects.

### **1.3.1. Properties of Carbon Dots**

#### **1.3.1.1 Optical Absorption**

CDs demonstrate strong optical absorption in the ultraviolet (UV) region, extending into the visible light spectrum. This absorption is mainly due to the  $\pi$ - $\pi^*$  transitions of the aromatic  $\text{sp}^2$  carbon domains and the  $n$ - $\pi^*$  transitions from the carbon core's C=O bonds, or potentially from surface state transitions that involve the electron's lone pairs (Khairul Anuar et al., 2021; Mohammad-Jafarieh et al., 2021).

#### **1.3.1.2 Luminescence Features**

The fluorescence of CDs is known to vary with the excitation wavelength, attributed to the selection of different-sized nanoparticles (quantum size effect) or varied emissive sites on the CDs' surface (Kaviya, 2018). Key factors influencing CDs' fluorescence include surface states, quantum size effects, the presence of conjugated  $\pi$ -domains, exciton trapping, edge defects, and the existence of multiple luminescent centers (Ding et al., 2020). Quantum confinement significantly contributes to the tunable luminescence of CDs, where the electronic and optical properties are dictated by the dimensions of the nanoparticles. This confinement leads to discrete electronic states, which alter the absorption and emission properties of the CDs. As the size of the CDs

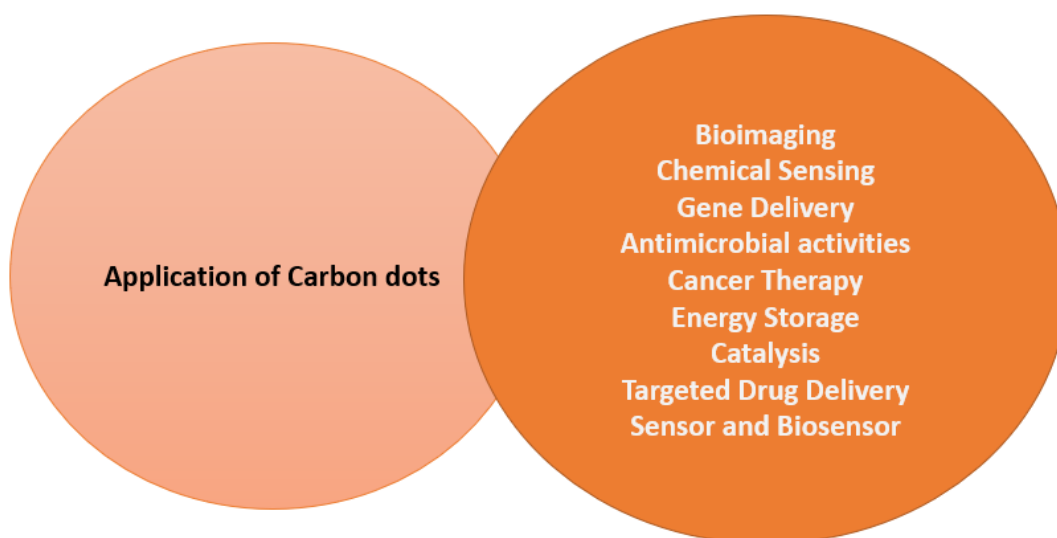
approaches or falls below the exciton Bohr radius, the energy difference between the valence and conduction bands increases, leading to a higher energy emission upon recombination of electron-hole pairs. Environmental factors such as temperature, pH, and concentration significantly affect their luminescence. The fluorescence efficiency or quantum yield (QY) of CDs, indicating the proportion of absorbed to emitted photons, is assessed against well-characterized standard references (Würth et al., 2015).

#### **1.3.1.3 Electrochemical Behavior**

CDs are noted for their excellent electrochemical performance, attributable to unique electron-transfer capabilities and extensive surface area. The presence of various surface functional groups, robust intra-molecular connections, and doped atoms (like N, P, S, B) enhances electrocatalytic activities by modifying the electronic properties of carbon through charge redistribution (Mansuriya & Altintas, 2021; X. Wang et al., 2019; Yadav et al., 2023). CDs exhibit size-dependent semiconductivity, a phenomenon where the electronic and optical properties change significantly with particle size due to quantum confinement effects. As the size of the CDs decreases, the band gap increases, leading to a blue shift in the absorption and fluorescence spectra.

#### **1.3.2 Utilization of Carbon Dots**

CDs being exclusively carbon-based composition, present lower toxicity compared to other nanomaterials and have a diminutive size that facilitates cellular entry, broadening their potential for biological applications. They are increasingly recognized for roles in photocatalysis, energy conversion and storage, chemical sensing, and various biomedical uses including biosensing, bioimaging, gene and drug delivery, and catalysis in addressing cancer, neurodegenerative, and infectious diseases. Their advantages over metal-based quantum dots include better water solubility, easy functionalization, chemical stability, low toxicity, excellent biocompatibility, and fluorescence, which broadens their application scope, especially in biomedical fields (Bartkowski et al., 2024; Cui et al., 2021; Z. Feng et al., 2021; J. Liu et al., 2020).



**Figure 3:** Applications of Carbon Dots

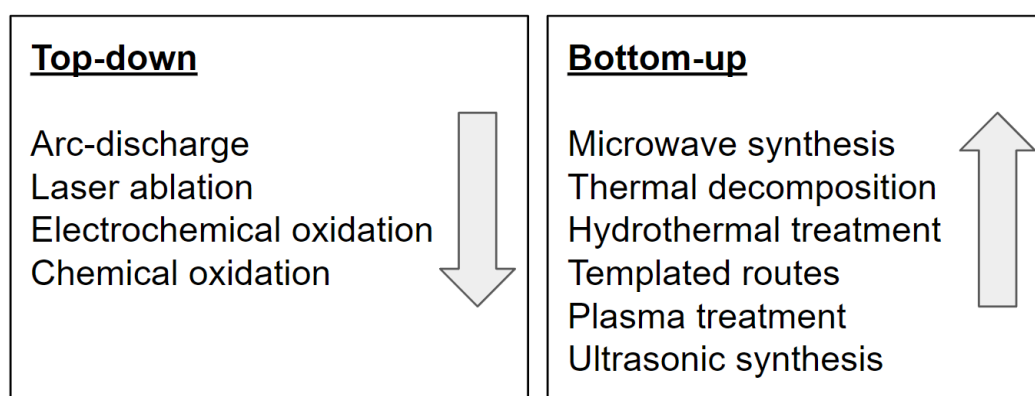
This plot illustrates the wide-ranging applications of carbon dots, including their use in bioimaging, chemical sensing, gene delivery, antimicrobial treatments, cancer therapy, energy storage, catalysis, drug delivery, and sensor and biosensor development. In this study, antimicrobial activity of synthesized CDs has been studied.

### **1.3.3 Antibacterial Activity of Carbon Dots**

CDs penetrate bacterial cells, attributed to their small size, positive charge, amphiphilic nature, and surface functional groups. They can be engineered for microbial detection by attaching antibiotics or integrating with other nanoparticles like hydrogels and magnetic particles (Lin et al., 2022). Their antibacterial action is multifaceted, involving ROS generation surpassing bacterial antioxidant defenses, biofilm inhibition, preservation of antibiotic properties, and primarily causing cell wall damage (Yu et al., 2023). The antibacterial mechanism includes ROS induction on bacterial surfaces under light exposure, disrupting the bacterial cell wall/membrane, initiating oxidative stress leading to DNA/RNA damage, altering or inhibiting essential gene functions, and causing oxidative harm to internal biomolecules like proteins (Lin et al., 2022; Yu et al., 2023).

## 1.4 Synthesis of Carbon Dots

The creation of CDs can be achieved through various methodologies, categorized mainly into top-down and bottom-up approaches (Cui et al., 2021). Top-down methods include arc discharge, laser ablation, and acidic oxidation, while bottom-up strategies encompass combustion, microwave pyrolysis, hydrothermal/solvothermal synthesis, and electrochemical techniques. However, certain methods like laser ablation and ion beam radiation come with the drawback of requiring expensive, energy-intensive equipment. Moreover, chemical oxidation, when involving strong acids, deviates from eco-friendly principles, highlighting the need for more sustainable, cost-effective synthesis routes.



**Figure 4:** CDs preparation methods.

In environmentally and sustainable synthesis methods, hydrothermal synthesis has emerged as a preferred route for CD production (Alsaiari et al., 2023; Altammar, 2023). This approach uses natural, renewable carbon sources, offering a clean and accessible alternative. Various natural materials such as fruit juices, plant extracts, and waste biomass have been explored as carbon precursors for CDs, underscoring the versatility and ecological advantages of green synthesis (Khairol Anuar et al., 2021).

### 1.4.1 Hydrothermal Method

Hydrothermal synthesis is a much common process to synthesize carbon dots. This is based on formation of quantum sized particles from high-temperature water solutions under high pressure. The core apparatus used in this method is a robust steel pressure vessel, known as an autoclave, where the raw material (nutrient) is introduced along with water. A main aspect of this process is the maintenance of a temperature gradient

within the growth chamber; the nutrient dissolves at the higher temperature end and crystallizes at the lower temperature end, fostering the growth of the desired sized particles (Kafle, 2020).



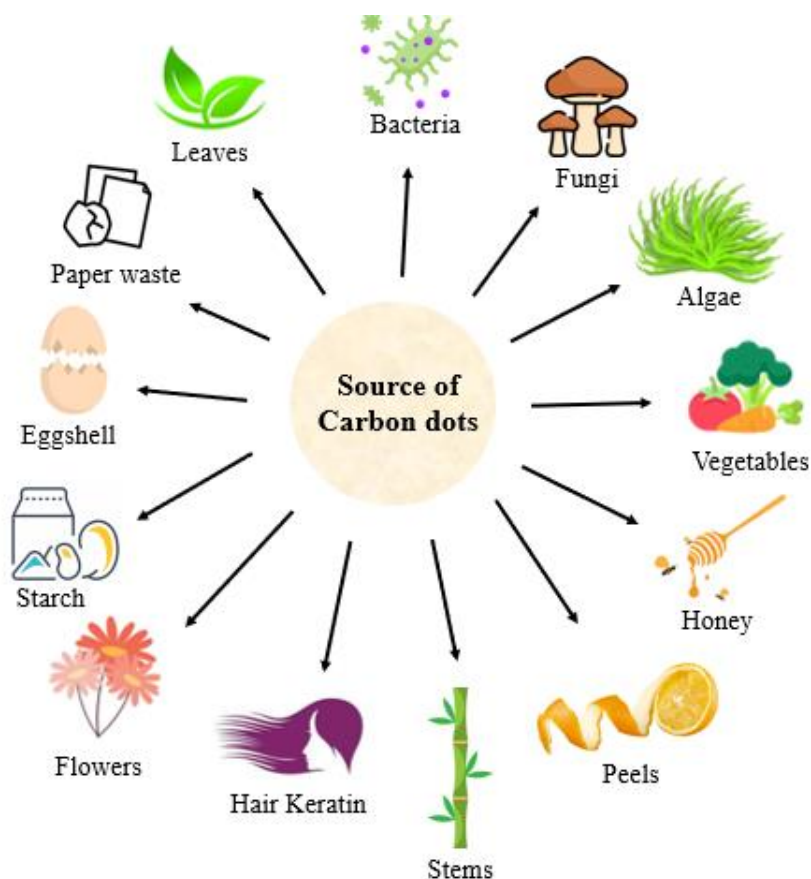
*Figure 5: Hydrothermal Synthesis Instrument*

Hydrothermal synthesis stands out among the best methods for its simplicity, efficiency, and ability to use a wide range of natural starting materials (Jorns & Pappas, 2021). This process involves the reaction of a precursor solution under high temperature and pressure conditions in a sealed container, typically a Teflon-lined autoclave (S.-H. Feng & Li, 2017). The method allows for the low-temperature conversion of biomass into highly luminescent carbon dots, leveraging the inherent organic compounds present in the natural sources.

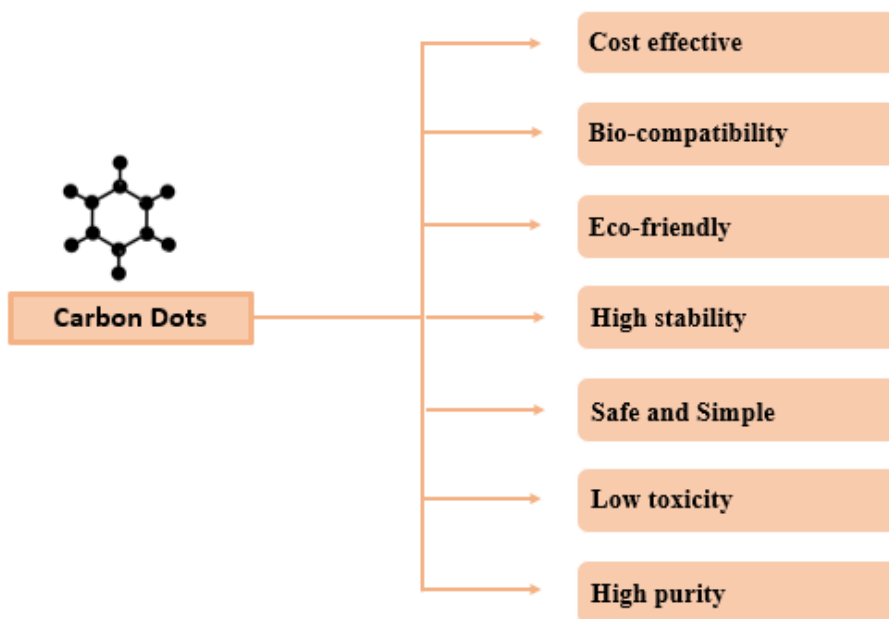
### **1.5 Sources of carbon Dots**

CDs can be derived from a wide range of natural resources, which are grouped into categories based on their origin. Following materials categorized as in figure 6 reflect the wide adaptability and potential of CDs for sustainable use in technological applications.

<b>Vegetables</b>	<b>Fruits</b>	<b>Waste material</b>	<b>Food &amp; Drink</b>	<b>Animal material</b>	<b>Leaves</b>	<b>Human material</b>
Cabbage Cucumber Curcumin Ginger Carrot Sweet pepper Onion Potato Tomato Radish	Banana Coconut Ginkgo fruit Gooseberry Kiwi Avakado Lemon Prickly pear Tamarind Watermelon	Cornstalk Waste food Peanut shells Waste Paper Kitchen waste Frying oil Sugarcane Onion shell Lemon peel	Butter oil Cola Nescafe Bread Carrot juice Molasses Soya milk Caramel Sugar	BBQ meat Chicken egg Shrimp egg Crabshell Pig skin Silk cocoons Wool	Leave bagasse Coriander leave Mushroom Ginkgo leave Pollen Potted plants Tulsi leave	Hair keratin Human urine



**Figure 6:** Synthesis of carbon dots, categorized by origin including vegetables, fruits, waste material, food & drink, animal material, leaves, and human-derived substances

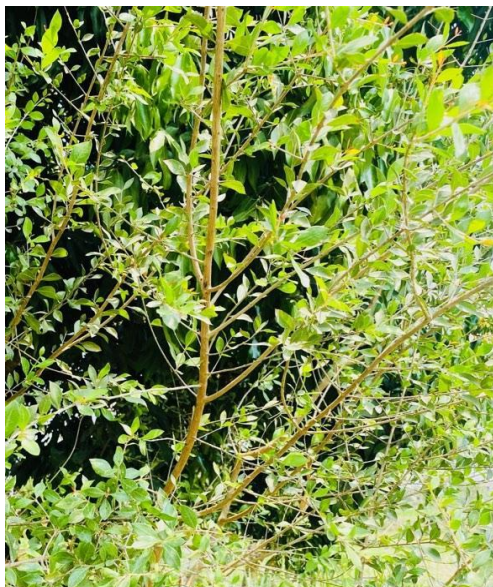


**Figure 7:** Advantages of CDs

### 1.5 1 *Lawsonia inermis* as a Carbon Source

*Lawsonia inermis*, widely recognized as henna plant, holds a place in cultural and medicinal realms and also presents a novel avenue in the field of nanotechnology, particularly in the synthesis of carbon dots (Mary Alex et al., 2020). This plant, heralded for its traditional use in dyeing and therapeutic applications, embodies a treasure trove of phytochemicals, including lawsone, flavonoids, tannins, and phenolic compounds, among others. The richness in natural dyes and organic compounds makes *Lawsonia inermis* an exemplary candidate for carbon dot production, combining the principles of sustainability with advanced technological applications (Mary Alex et al., 2020).

CDs synthesis from this plant is eco-friendly (Mary Alex et al., 2020), cost-effectiveness as it is a widely cultivated plant with minimal requirements for growth and maintenance (Farzin & Abdoos, 2021). This advantage is pivotal in scaling up synthesis processes without imposing significant financial or environmental burdens. The biocompatible CDs obtained from this plant can have huge beneficial biomedical applications. The taxonomic classification is given below.



*Figure 8: Lawsonia inermis*

### **Taxonomic classification**

Kingdom: Plantae  
Division: Magnoliophyta  
Class: Magnoliopsida  
Order: Myrtales  
Family: Lythraceae  
Genus: *Lawsonia*  
Species: *inermis*  
Common Name: Henna/Mehendi

*Lawsonia inermis* offers a simple, eco-friendly, and safe approach for synthesizing high-stability and high-purity carbon dots. Its cost-effectiveness and biocompatibility underscore the low toxicity and substantial advantages these CDs have, holding promise for extensive applications across diverse scientific fields.

## **1.6 Research objectives**

### **1.6.1 General Objectives**

The general objectives of this study is to synthesize CDs using *Lawsonia inermis* leaves extract and study its antimicrobial activities.

### **1.6.2 Specific Objectives**

The specific objectives of this study can be figured out as follows:

1. Synthesis of CDs by hydrothermal using *Lawsonia inermis* leaves extract as nutrient.
2. Characterization of synthesis CDs by using UV Spectroscopy, FTIR and XRD.
3. Studying band gap and zeta potential of carbon dots.
4. Studying antimicrobial activities of synthesized carbon dots against selected bacteria and fungus.

## CHAPTER 2

### LITERATURE REVIEW

The exploration of CDs for biomedical applications, particularly their antimicrobial properties, has garnered significant attention in recent years. This surge in interest is largely due to the unique properties of CDs, including their quantum size effect, high surface area, and the ease of functionalization. Various carbon sources have been utilized for CD synthesis, each contributing to the diversity in the properties and applications of the resulting nanoparticles. Among these, plant extracts have emerged as promising green precursors due to their sustainability and the additional functional benefits derived from phytochemicals.

In the 2020 study by Boobalan et al. CDs were synthesized from oyster mushrooms using a hydrothermal process. These C-dots proved effective in detecting toxic Pb<sup>2+</sup> ions, with detection and quantification limits at 58.63  $\mu$ M and 177.69  $\mu$ M, respectively. Additionally, they served as fluorescent probes for DNA recognition, indicating potential in biosensing. The study also demonstrated the C-dots' antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Lastly, these CDs exhibited anticancer properties against MDA-MB-231 breast cancer cells (Boobalan et al., 2020).

The work of Gao et al. offers a different perspective by utilizing ampicillin as a precursor in the hydrothermal synthesis of CDs. Their findings revealed a strong antibacterial effect against *S. aureus* and *L. monocytogenes*, showcasing the potential of CDs in combating bacterial infections under visible light. This study emphasizes the antimicrobial capabilities of CDs and also their application in photodynamic therapy, offering a dual function that could significantly enhance the efficacy of bacterial eradication strategies (Gao et al., 2020).

Li et al.'s research further expands the scope of CDs in antibacterial and antifungal domains. By synthesizing CDs from vitamin C through an electrochemical process, they demonstrated the broad-spectrum antimicrobial activity of CDs, effectively inhibiting both gram-positive and gram-negative bacteria, as well as fungi like *Rhizoctonia solani* and *Pyricularia grisea*. This study highlights the versatility of CDs, suggesting their

potential in diverse biomedical applications, from combating bacterial infections to addressing fungal diseases (Li et al., 2018).

Muktha et al. explored the antimicrobial and cytotoxic properties of CDs synthesized from pomegranate and watermelon peels using the microwave method. Their findings underscore the feasibility of utilizing waste biomass as a sustainable carbon source and also demonstrate the effective antibacterial and antifungal activities of the resulting CDs. This approach contributes to waste valorization and also opens new avenues for the development of eco-friendly antimicrobial agents (Muktha et al., 2020).

The literature shows a myriad of synthesis methods and carbon sources for producing CDs, each contributing unique properties to the nanoparticles. For example, studies utilizing *Carica papaya* juice and Henna plant extracts have shown effective antibacterial activities against common pathogens (Khairol Anuar et al., 2021; Parhama et al., 2023; Sahu & Sahoo, 2024). Similarly, the use of rosemary leaves and tea leaf extract for CD synthesis further exemplifies the versatility of plant-based precursors in producing CDs with potent antimicrobial properties (Eskalen et al., 2021).

Recent advancements in the field of CDs have emphasized their potential in a wide range of biomedical applications, driven by their unique optical properties, biocompatibility, and ease of functionalization. Notably, a 2019 study by Anwar et al. in the *ACS Applied Bio Materials* journal detailed the synthesis, optical properties, and various biomedical applications of CDs, marking significant progress in their development, particularly for nanomedicine (Anwar et al., 2019).

Further exploration into the analytical and biomedical capabilities of CDs was presented by Ross et al. (2020), who reviewed their use as diagnostic tools and theranostic agents. This work highlighted the versatility of CDs in sensing applications, particularly for detecting toxic metal ions and their substantial role in cell labeling processes, showcasing their broad applicational scope (Ross et al., 2020).

Ghosal and Ghosh (2019) in their publication in *Materials Science and Engineering: C*, have elaborated on the next-generation platform that CDs offer for biomedical applications. They reviewed the synthesis methods, detailed the biocompatible properties, and discussed various biomedical applications of CDs, further underscoring the adaptability of CDs to meet diverse medical needs (Ghosal & Ghosh, 2019).

In addition, the work by Su et al. (2020) in *Materials Chemistry Frontiers* explored the significant potential of CDs in biomedical fields, focusing on their optical properties

and various applications, including drug delivery and bioimaging. This comprehensive review not only highlighted the current capabilities of CDs but also pointed towards future directions in research and development (Su et al., 2020). Behi et al. (2022) provided a comparative study of traditional quantum dots and CDs in *Nanoscale Advances*, illustrating the advantages of CDs over traditional options due to their less toxic nature and similar functional versatility. This study emphasized the burgeoning interest in CDs as safer alternatives for biomedical applications (Behi et al., 2022). And in recent years, hydrothermal methods have significantly piqued interest to synthesize carbon over different carbon sources (Table 1).

Despite this research, gaps remain, particularly in the context of the origin and composition of the plant materials used (Barman & Patra, 2018; He et al., 2022; M. L. Liu et al., 2019). The biochemical profile of a plant can significantly influence the properties of the synthesized CDs, yet this aspect remains underexplored. Specifically, *Lawsonia inermis*, known for its medicinal properties and rich phytochemical content, presents a promising candidate for CD synthesis. However, the influence of its specific origin, such as from Banganga Municipality ward No 8, Kapilvastu, Nepal, on the properties and antimicrobial efficacy of the resulting CDs has not been thoroughly investigated. This locality, with its unique climate and soil conditions, may impart distinctive phytochemical profiles to *Lawsonia inermis*, potentially affecting the synthesis and functional properties of CDs derived from it. Addressing this research gap could provide new insights into the role of geographic origin in the green synthesis of CDs, offering a better understanding of their antimicrobial mechanisms and broadening their application spectrum in biomedicine.

**Table 1:** CDs synthesis from various carbon sources and synthetic methods.

CARBON SOURCES	SYNTHETIC METHOD	CHARACTERIZATION	APPLICATIONS	REFERENCE
Onions	Autoclaving method	TEM, XPS, FTIR, DLS, XRD, TGA, Raman spectroscopy (RS), and FS	Fe <sup>3+</sup> detection and multicolor cell imaging	Bandi et al., 2016
Prawn shells	Autoclaving method	UV-vis, FTIR, FS, EDX, XRD, TEM	Cu <sup>2+</sup> detection	Gedda et al., 2016
Carrot juice	Hydrothermal	TEM, XRD, FTIR, XPS, UV-vis, FS	Cytotoxicity and cell imaging	Y. Liu et al., 2017

<b>Rose-heart radish</b>	Hydrothermal	TEM and HRTEM, FTIR, UV-vis, XPS and FS	Fe <sup>3+</sup> detection and cell imaging	W. Liu et al., 2017
<b>Peach juice</b>	Hydrothermal	XRD, FTIR, XPS, FESEM, EDX, HRTEM	Bioimaging	Atchudan et al., 2018
<b>Vitamin 'C'</b>	One-step Electrochemical	TEM, HRTEM, XPS, UV-vis, FTIR	Antibacterial activity, Antifungal activity	Li et al., 2018
<b>Coconut leaves</b>	Hydrothermal	FTIR, UV-vis, TEM, and XRD	Biosensing and antibacterial activity	Joshi et al., 2018
<b>Beer yeast powder</b>	Hydrothermal	FTIR, XPS, UV-vis, SEM	Antibacterial activity	Gao et al., 2019
<b>Osmanthus fragrans</b>	Hydrothermal	UV-vis, FS, TEM, XRD, FTIR, XPS, EDS	Cytotoxicity and cell imaging, Fe <sup>3+</sup> and Ascorbic acid detection	M. Wang et al., 2019
<b>Sodium citrate and Urea</b>	Hydrothermal	FTIR, EDS, TEM, UV-vis, Electrochemical impedance spectroscopy, Fluorescence lifetime measurements	As a sensitizing agent for fabrication of dye sensitized solar cell.	Ghann et al., 2019
<b>Sugarcane industrial waste</b>	Hydrothermal	XRD, XPS, FTIR, UV-vis, HRTEM	Antibacterial activity	Pandiyar et al., 2020
<b>Ampicillin</b>	Hydrothermal	TEM, UV-vis, XPS, SEM, FTIR	Antibacterial activity	Gao et al., 2020
<b>Pomegranate and Watermelon peels</b>	Microwave	FTIR, HRTEM, FS, Raman spectroscopy, UV-vis	Antimicrobial activity and cytotoxicity	Muktha et al., 2020
<b>Turmeric leaves</b>	Hydrothermal	TEM, UV-vis, Fluorescence Spectroscopy (FS), FTIR, XPS	Antibacterial activity	Saravanan et al., 2021
<b>Elettaria cardamomum</b>	Ultrasonication method	XRD, FTIR, RS, UV-vis, photoluminescence spectroscopy	As a photocatalyst in dye degradation	Zaib et al., 2021
<b>Turmeric</b>	Hydrothermal	FETEM, XPS, XRD, FTIR	Zeta potential, antibacterial, antioxidant	Roy et al., 2022s

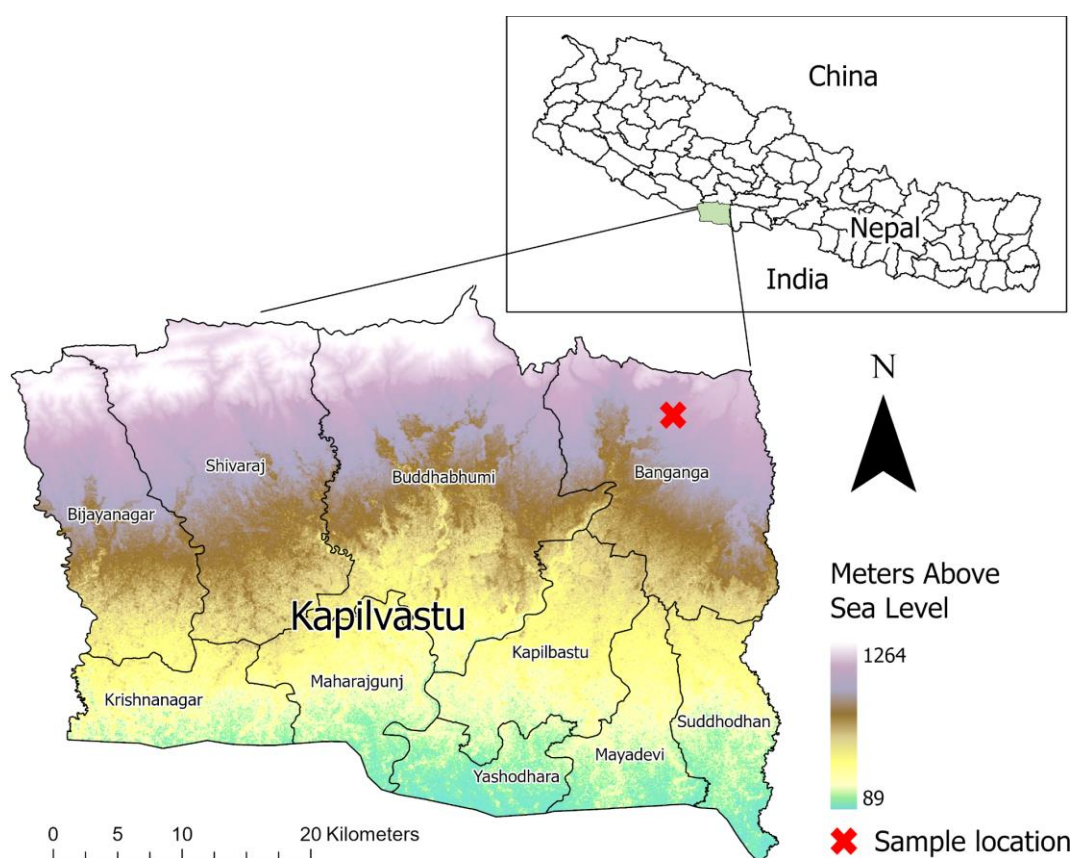
Table 1 is a compilation of various relevant studies focusing on the synthesis, characterization, and applications of CDs using diverse carbon sources. Hydrothermal synthesis appears to be the prevailing method, suggesting a growing interest in this approach due to its efficiency and eco-friendliness. Characterization techniques are comprehensive, including UV-vis, FTIR, TEM, and more, affirming the multifaceted nature of CDs. Applications span from antibacterial and anticancer activities to sensing and cell imaging, highlighting the versatility of CDs in various fields.

## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1 Materials Collection

Leaves of *Lawsonia inermis* were collected from the lush vegetation of Banganga Municipality ward No 8, Kapilvastu, Nepal (Longitude: 27° 41' 37" latitude: 27° 41' 37", Altitude: 107 m). The selection of this specific location was motivated by its rich flora and favorable climatic conditions that ensure the growth of *Lawsonia inermis* with a good profile of biochemical constituents. These naturally thriving plants provide an organic, untreated source of leaves, rich in essential phytochemicals for the synthesis of CDs.



**Figure 9:** Map of the study area and location of collected sample. A marked red "X" indicates the specific location within the Banganga area where the sample was collected.

#### 3.2 Preparation of Extract

Aqueous extract of *Lawsonia inermis* leaves was prepared by cold percolation method. Initially, the collected leaves were shade-dried for a period to ensure the removal of all moisture, thereby preserving the integrity of the phytochemicals. Then dried leaves were finely ground into a powder. 4 g of this powdered material was then soaked in 140

mL of distilled water, in conical flasks. These flasks were placed on a Magnetic Stirrer and Magnet is kept in the conical flask and stirring is done for a duration of 30 hours to ensure thorough extraction of the phytochemicals. The mixture was then filtered using Whatman Filter Papers to obtain a clear filtrate or to remove impurity, which was then kept in a beaker at room temperature for further use.



**Figure 10:** Preparation of Lawsonia inermis Extract for Carbon Dot Synthesis. a) Lawsonia inermis leaves powder, b) Extraction process in progress, c) Filtration of the extract

### 3.3 Hydrothermal Synthesis of Carbon Dots

The hydrothermal synthesis of CDs was initiated by taking in two one is 50 mL and another is 100 mL of hydrothermal and only 70% of total of hydrothermal was kept inside the Teflon Chamber and in 50 mL of hydrothermal only 30 mL extract was kept inside and In 100 mL of Hydrothermal 70 mL of solution was kept inside the Teflon chamber. This mixture was then subjected to a Hydrothermal process Kept inside the Muffle furnace at 180 °C for 12 hours, resulting in a solution that is brown and then carbon dot solution is filtered in Whatman filter paper for clear solution of carbon dots and its, indicating the formation of CDs. Following this, the solution underwent centrifugation at 13000 rpm, 12 min/per round to separate the supernatant from the precipitate. The clear supernatant was then subjected to a dialysis process for 48 hours using distilled water to purify the CDs. Upon exposure to a UV lamp, the purified solution exhibited greenish fluorescence at 365 nm, confirming the successful synthesis of CDs. The solution was then freeze-dried at -70 °C for 24 hours to obtain the CDs in solid form, which were stored in.



(a)

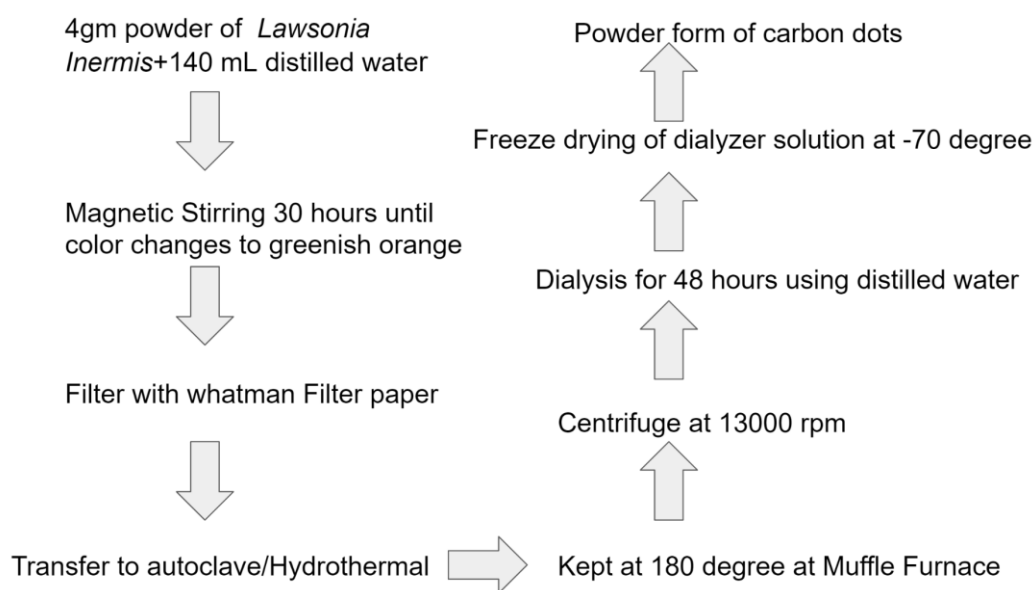


(b)



(c)

**Figure 11:** a) Hydrothermal synthesis, b) Centrifuge, c) Dialysis



**Figure 12:** Flow diagram sheet diagram of hydrothermal synthesis of carbon dots



**Figure 13:** Freeze dryer

### **3.4 Characterization of Synthesized Carbon Dots**

#### **3.4.1 UV-Visible Spectroscopy**

The optical properties of the synthesized CDs were first evaluated using UV-Visible spectroscopy, a technique for understanding the interaction between light and matter at the nanoscale. To perform this analysis, an aqueous solution of the CDs was prepared, ensuring that the concentration was optimal for absorption measurements. The spectra were recorded in the 200-1100 nm wavelength range using a single-beam UV-visible spectrometer. Measurement was carried out at the Department of chemistry, Amrit Campus, Kathmandu using a single beam UV- visible spectrometer. This wide range allowed for the observation of any optical absorption features characteristic of CDs, including the identification of the quantum confinement effect. The quantum confinement effect is a phenomenon where the electronic properties of nanoparticles differ significantly from those of bulk materials due to their size. For CDs, this effect manifests in unique absorption peaks, which are indicative of their size, shape, and surface chemistry. The specific wavelengths at which these peaks occur provide valuable insights into the electronic structure and optical behavior of the CDs, including potential applications in bioimaging and phototherapy.

#### **3.4.2 FTIR Spectroscopy**

Fourier Transform Infrared (FTIR) Spectroscopy was utilized to identify the functional groups present on the surface of the CDs, offering clues to their chemical bonding and composition. For this analysis, dry samples of the CDs were prepared to ensure that water or other solvents did not interfere with the spectral data. The samples were analyzed using Perkin Elmer 10.6.2 spectrometer at the Department of Chemistry, Amrit Campus, Kathmandu, Nepal in Attenuated Total Reflectance (ATR) mode, covering a spectral range of 450-4000  $\text{cm}^{-1}$  with the scanning interval of 4  $\text{cm}^{-1}$ . This technique is particularly sensitive to the vibrational modes of chemical bonds, making it ideal for detecting organic functional groups such as hydroxyl (-OH), carbonyl (C=O), and amine (-NH<sub>2</sub>) groups, among others. These functional groups play a significant role in the solubility, biocompatibility, and reactivity of CDs, making FTIR a vital tool in their characterization. By comparing the observed spectra to known standards, it was possible to confirm the successful incorporation of phytochemicals

from the *Lawsonia inermis* extract into the CDs, providing a foundation for understanding their interactions with biological systems.

### **3.4.3 Zeta Potential Measurement**

Finally, the colloidal stability and surface charge of the CDs were assessed through zeta potential measurements. This parameter is a key indicator of the potential stability of colloidal dispersions, with values further from zero (both positive and negative) suggesting greater colloidal stability. 5mg of sample with 20mL distilled water is taken and 3 min sonicator is done and then solution was kept in zeta cell and with the help of two electrode zeta potential was measured using a Horiba instrument at Nepal Academy of Science and Technology (NAST) Lalitpur Nepal with registration number #1764. The zeta potential provides insights into the surface chemistry and charge distribution of the CDs, which are important factors in their interaction with biological molecules and cells. A high zeta potential can prevent the aggregation of CDs, ensuring their uniform dispersion in solutions, which is for consistent performance in bioimaging, sensing, and drug delivery applications.

### **3.4.4 Band Gap Measurement**

The determination of the band gap of the carbon dots was performed to understand their electronic properties, which is for applications in optoelectronics and photonics. The band gap is the energy difference between the top of the valence band and the bottom of the conduction band in semiconductors. It defines the energy range in which no electron states can exist and is thus vital in determining the wavelengths of light that a material can absorb or emit. In practice, the band gap measurement of CDs involves analyzing the absorption edge in the UV-Vis spectrum and applying the Tauc plot method, where the absorption coefficient is plotted against the photon energy to extrapolate the band gap energy value. It was conducted at the Pulchowk Campus.

### **3.5.5 Phytochemical test**

A series of phytochemical tests were conducted to identify the presence of various bioactive compounds in the *Lawsonia inermis* leaf extract used for synthesizing carbon dots was conducted at Department of Chemistry, Amrit Campus, Kathmandu. These tests qualitatively detect the presence of alkaloids, flavonoids, tannins, saponins, and other phytochemicals. The significance of these tests lies in confirming the successful

extraction of these compounds, which are hypothesized to play a role in the formation and stabilization of carbon dots during the synthesis process. The presence of specific phytochemicals can also impart unique properties to the CDs, such as enhanced fluorescence or increased biocompatibility.

### **3.5 Antibacterial Activity**

The antibacterial properties of the carbon dots synthesized from *Lawsonia inermis* leaves were assessed through the agar well diffusion method at Himalaya Research Institute of Biotechnology, Srijananagar, Bhaktapur. In this process, both gram-positive bacteria, including *Staphylococcus aureus* and *Bacillus subtilis*, and both gram-negative bacteria, such as *Escherichia coli* and *Klebsiella Pnuemoniae* and one fungal *C.albicans* were cultivated in liquid broth media. Prepared Mueller-Hinton Agar (MHA) plates were inoculated with these bacterial cultures, and wells were punctured into the agar to introduce the carbon dot samples. Following incubation, the inhibition zones were measured, providing a quantitative analysis of the carbon dots' antimicrobial efficiency.

#### **3.5.1 Protocol for Antimicrobial Test**

##### **3.5.1.1 Preparation of Microbial Culture Media**

The preparation involved dissolving 13 g of LB powder in 1 L of distilled water, followed by sterilization through autoclaving at 121 °C and 15 psi for 25 minutes. After cooling to a manageable temperature, the media was allotted into sterilized falcon tubes and used to culture bacterial strains for 24 hours.

##### **3.5.1.2 Preparation of MH Media Plates and Antimicrobial Assay**

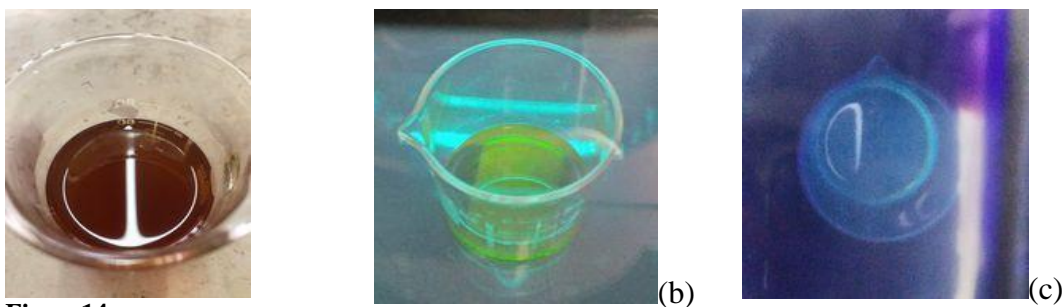
MH agar was prepared by mixing 39 g of MH agar powder with 1 L of water, undergoing a similar autoclaving process as the LB media. Once cooled, the MH agar was poured into Petri dishes and allowed to solidify. The bacterial seed cultures were uniformly spread onto these plates. Wells were then created on the surface of the agar using a sterile technique. Into these wells, aliquots of the carbon dot sample (100 µL, at a concentration of 100 mg/mL) and a standard kanamycin solution (5 mg/mL, 10 µL) were introduced. The inoculated plates were incubated at 37 °C for 24 hours, after which the zones of inhibition were observed and measured to evaluate the antimicrobial activity of the samples.

## CHAPTER 4

### RESULTS AND DISCUSSION

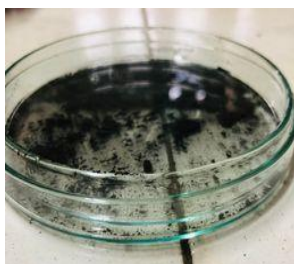
#### 4.1 Visual Characterization of CDs

The hydrothermal process, utilizing an aqueous extract of *Lawsonia inermis* leaves, facilitated the successful synthesis of carbon dots. From an initial quantity of 24 g of *Lawsonia inermis* powder, approximately 0.2 g of carbon dots were synthesized. These carbon dots showed a characteristic deep green fluorescence under UV light at 365 nm, indicative of successful synthesis. The fluorescence observed is attributed to the  $n-\pi^*$  transitions and quantum confinement effects, which are influenced by the particle size, nitrogen content, level of surface oxidation, and the types of surface functional groups present on the carbon dots.



**Figure14:**

Photographic representation of carbon dots synthesized via the hydrothermal process from *Lawsonia inermis* leaf extract, demonstrating the material's appearance: a) under visible light, b) under short UV light at 254 nm, and c) under long UV light at 365 nm

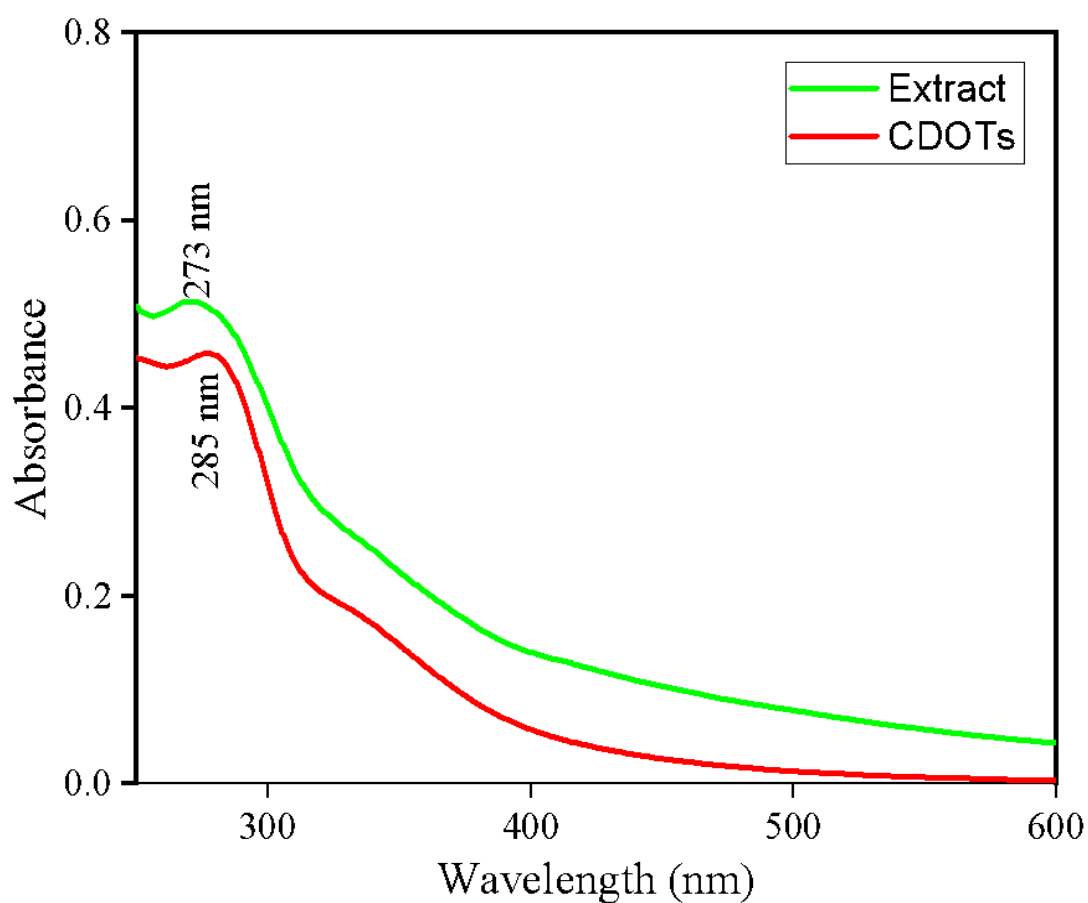


**Figure 15:** The CDs in solution form after the process of dialysis, indicating the removal of small molecules and impurities.

## 4.2 Characterizations of Synthesized Carbon Dots

### 4.2.1 UV-visible Spectroscopy

UV-visible spectroscopy revealed the optical properties of the synthesized carbon dots, with a significant absorption band at 200-1100 nm, which can be attributed to the  $\pi-\pi^*$  transition of the C=C bond and the  $n-\pi^*$  transition of the C=O bond. This confirms the successful synthesis of CDs and their expected optical behavior.



**Figure 16:** UV-Visible spectrum of the synthesized carbon dots and extract solution.

#### 4.2.2 Fourier Transform Infrared Microscopy (FTIR)

FTIR analysis identified various functional groups present in the CDs. The broad peak at  $3379.94\text{ cm}^{-1}$  is associated with the  $-\text{OH}$  and  $-\text{NH}$  groups' stretching vibrations. The peak at  $2937\text{ cm}^{-1}$  corresponds to C-H stretching vibrations, while the sharp absorption at  $1706\text{ cm}^{-1}$  is characteristic of C=O stretching vibrations. These spectral features confirm the diverse functional groups that contribute to the unique properties of the synthesized CDs.

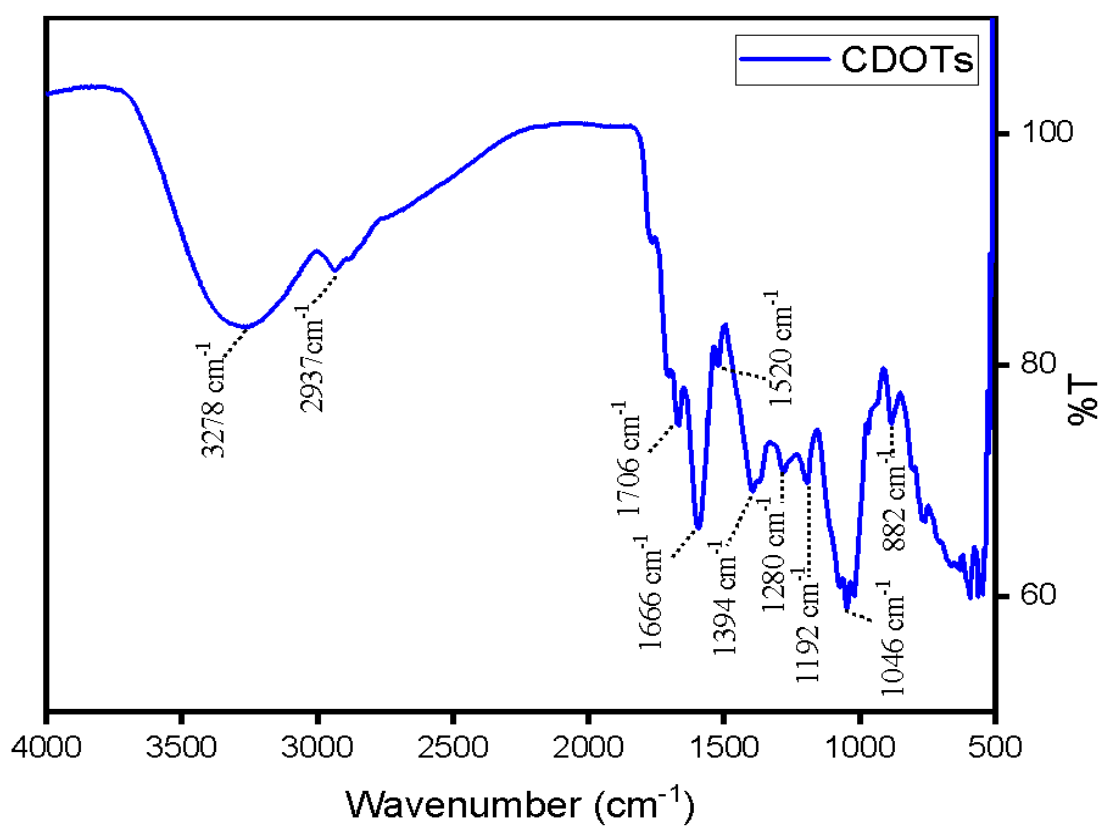
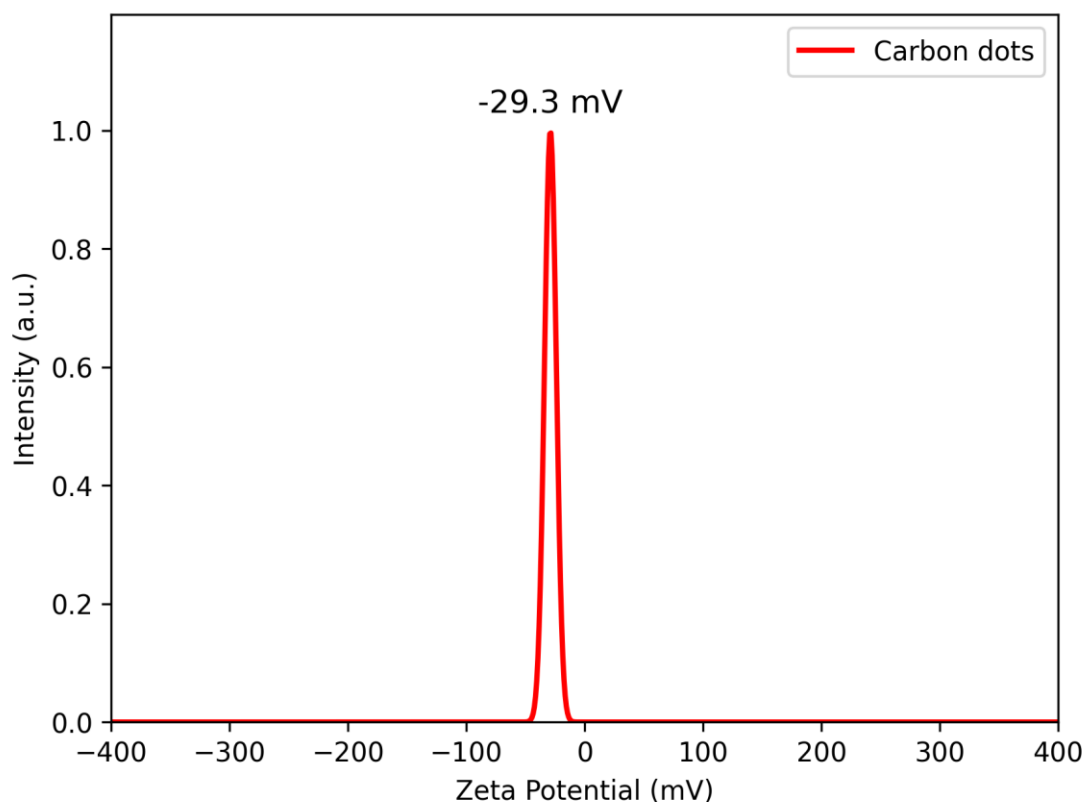


Figure 17: The FTIR spectrum of Carbon Dots

### 4.2.3 Zeta Potential Measurement

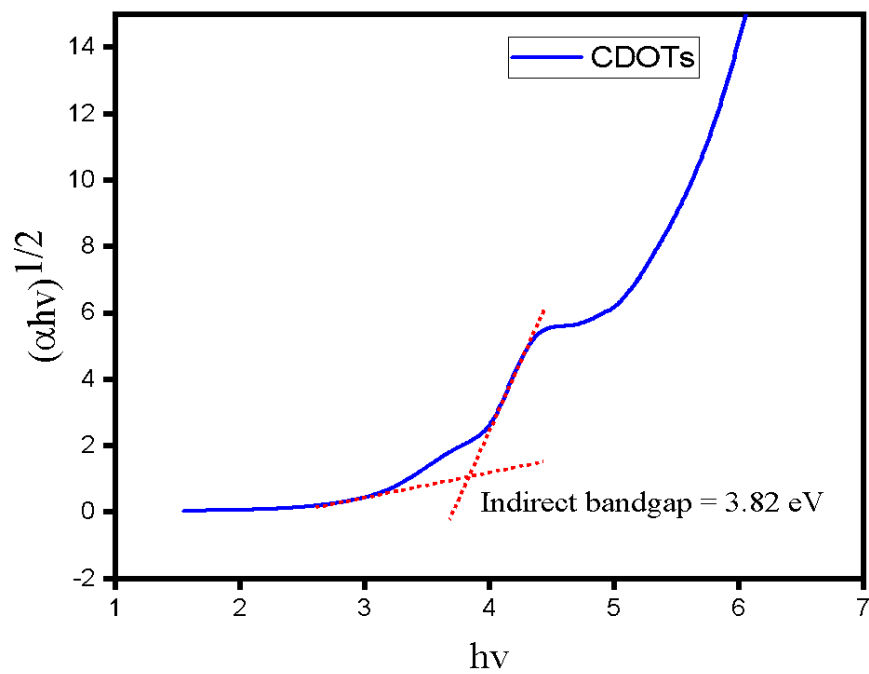
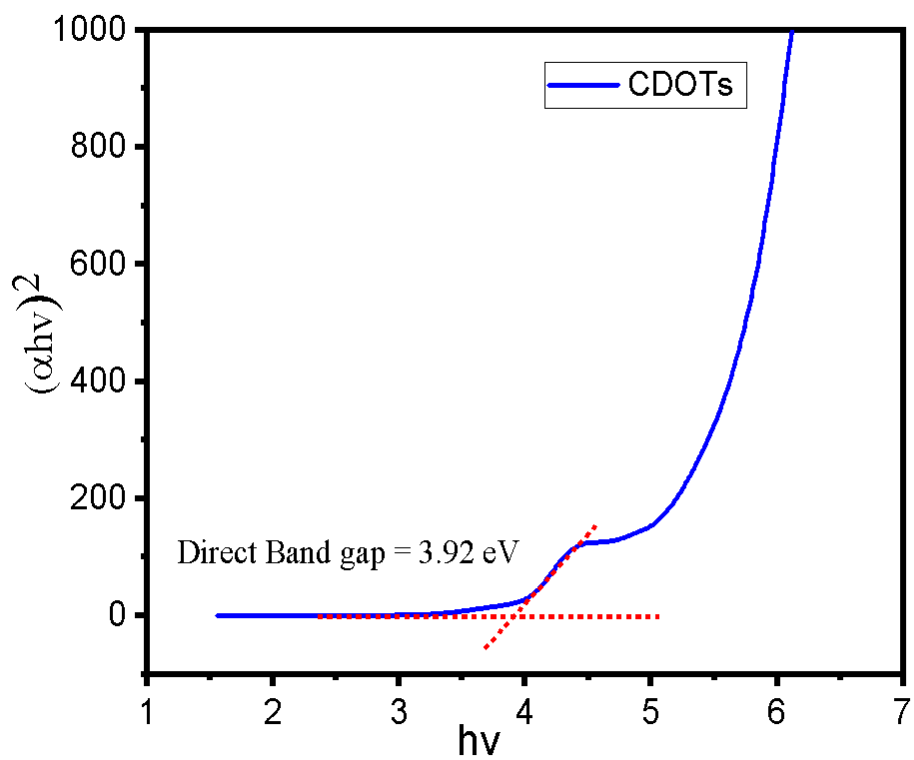
The zeta potential was measured to be  $-29.3\text{mV}$ , indicating the stability of the CDs in suspension and suggesting their surface charge characteristics, which are important for biological applications.



**Figure 18:** The zeta potential distribution of the CDs.

### 4.2.3 Band gap Measurement

Direct Band gap of *Lawsonia Inermis* synthesis carbon dots is  $3.92\text{eV}$ . Indirect Band gap of *Lawsonia Inermis* synthesis carbon dots is  $3.82\text{eV}$ .



**Figure 19:** Band Gap measurement of synthesized Carbon dots using Tauc plot

#### 4.2.4 Phytochemical Screening

A qualitative phytochemical screening of the *Lawsonia inermis* extract was conducted to detect the presence of various secondary metabolites. The identification relied on the manifestation of particular color changes upon chemical interaction. The screening results are tabulated as follows:

**Table 2:** Phytochemical analysis of extracts of Lawsonia Inermis

S. N	Class of Phytochemicals	Extract solution
1	Alkaloids	+
2	Flavonoids	+
3	Terpenoids	+
4	Steroids	+
5	Quinones	+
6	Saponins	-
7	Tannins	+
8	Phenolic Compounds	-
9	Carbohydrates	-
10	Protein	-

The table denotes the presence (+) or absence (-) of specific phytochemicals within the *Lawsonia inermis* extract, providing insight into the complex biochemical composition of the source material used for carbon dot synthesis.

#### 4.3 Antibacterial Activity

The antibacterial efficacy of the hydrothermally synthesized carbon dots was evaluated using the agar diffusion method. These carbon dots demonstrated inhibitory action against a range of bacterial strains, including both gram-positive and gram-negative bacteria, as well as fungal species. Specific zones of inhibition were recorded for *Staphylococcus aureus* (ATCC 6538P) and *Bacillus subtilis* (ATCC 6051), indicating the antibacterial potential of the carbon dots. Similarly, gram-negative bacteria such as *Escherichia coli* (ATCC 8739) and *Klebsiella pneumoniae* (ATCC 700603), and the fungal pathogen *Candida albicans* (ATCC 2091) exhibited inhibition zones demonstrating the broad-spectrum activity of the carbon dots.

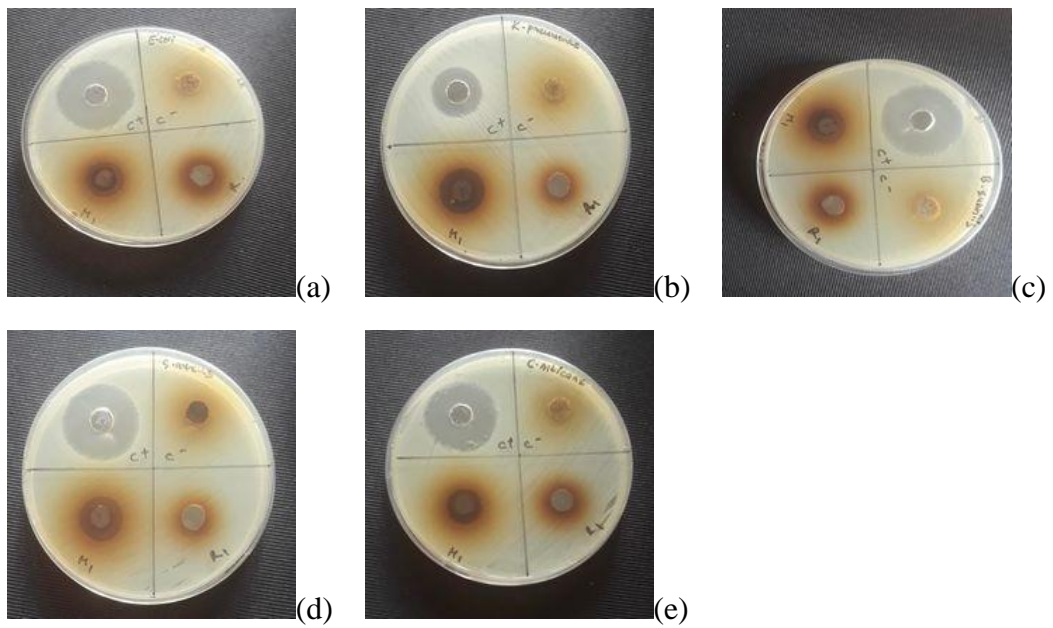
Kanamycin, a known antibiotic, was utilized as a positive control (c+) at a concentration of 5 mg/mL, while powdered *Lawsonia inermis* served as a negative control (c-). The comparison of inhibition zones between the controls and the carbon dot samples provides a clear measure of the synthesized carbon dots' antimicrobial potency.

In detail, the synthesized carbon dots presented an inhibition zone of [1.5] cm for *Staphylococcus aureus* and [1.8] cm for *Bacillus subtilis*, signifying an effective antibacterial action when compared to the [2.8] cm zone of the positive control and no inhibition from the negative control. For the gram-negative bacteria tested, *Escherichia coli* showed an inhibition zone of [1.5] cm and *Klebsiella pneumoniae* a zone of [1.6] cm. This activity was observed against a [2.8] cm and [1.8] cm inhibition by the positive control, respectively. The fungal strain *Candida albicans* exhibited an inhibition zone of [1.3] cm in the presence of the carbon dots, in contrast to a [2.6] cm zone by the positive control and no activity from the negative control.

These observations suggest that the antibacterial and antifungal capabilities of the carbon dots are significant. The mechanism behind the inhibitory action of the carbon dots is believed to involve the disruption of essential biological processes within the microbes, such as DNA/RNA synthesis and membrane integrity, primarily due to the nanoscale size of the carbon dots which facilitates their penetration into microbial cells. This mechanism has been supported by various studies that attribute the effective inhibition of bacterial growth to the physical and chemical interactions of the carbon dots with the microbial cells.

**Table 3:** Antibacterial Activity of Synthesized carbon Dots

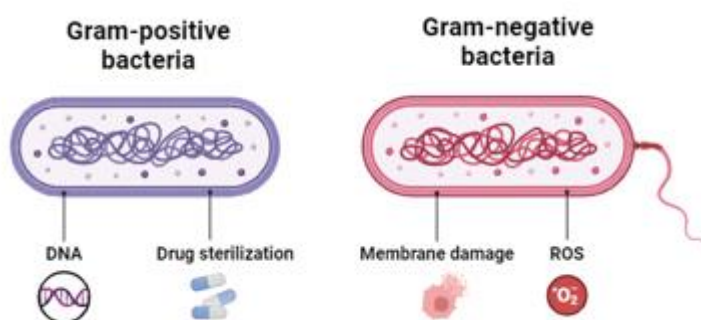
Bacterial strain	Reference culture	Type	Positive control (c+) cm	Negative control (c-) cm	Carbon Dots(H1)
<i>E. coli</i>	ATCC 8739	Gram-ve	2.8	0	1.5
<i>Klebsiella Pneumoniae</i>	ATCC 700603	Gram-ve	2.8	0	1.6
<i>B. subtilis</i>	ATCC 6051	Gram+ve	2.8	0	1.8
<i>Staphylococcus aureus</i>	ATCC 6538P	Gram+ve	2.8	0	1.5
<i>C. albicans</i>	ATCC 2091	Fungus	2.6	0	1.3



**Figure 20:** Petri dish assay results showing the antibacterial and antifungal activities of carbon dots synthesized from *Lawsonia inermis*: a) zones of inhibition against *Escheria coli*, b) against *Klebsiella pneumoniae*, c) *Bacillus subtilis*, d) *Staphylococcus aureus* and e) fungus *Candida albicans*

### 4.3.1 Mechanism of Antibacterial Activity of Synthesized Carbon Dots

The synthesized CDs demonstrate a potential antibacterial mechanism that is attributed to two primary actions: disruption of bacterial cell membranes through electrostatic interaction and the induction of oxidative stress via reactive oxygen species (ROS) when illuminated. These processes work in tandem to cause damage to the bacterial DNA and RNA, ultimately leading to cell death. The ROS generated in the presence of light may lead to the formation of singlet oxygen ( $^1O_2$ ) or superoxide anions ( $O_2^{\bullet-}$ ), or the CDs may act as catalysts, oxidizing other molecules.

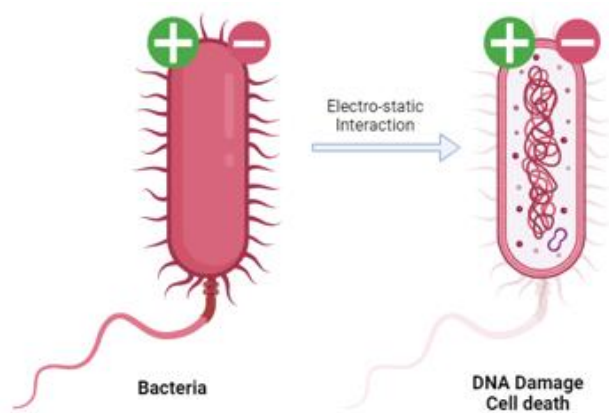


**Figure 21:** Different types of mechanism of gram positive and gram-negative bacteria

The antibacterial mechanisms of CDs highlight their ability to halt bacterial growth through a combination of physical membrane damage, metabolic disruption, oxidative stress, and photocatalytic effects. The prevalent mechanism is the oxidative stress caused by ROS, produced under light conditions, which significantly impacts bacterial survival. The negative zeta potential measured for these CDs indicates a high surface charge, consistent with the hydroxyl and NH groups identified in the FTIR spectrum. Moreover, the CDs exhibited a more pronounced antibacterial effect against gram-positive bacteria compared to gram-negative and fungal strains, suggesting a difference in the efficacy of CDs based on bacterial cell wall composition.

CDs can adhere to bacterial surfaces via electrostatic forces. Given their nanoscale size, CDs can penetrate bacterial cells post-adhesion. They are capable of destabilizing the DNA's helical structure, leading to growth inhibition. Furthermore, the surface charge of CDs influences their interaction with bacterial cell membranes, which may trigger various responses, including cell death. As suggested by some studies, positively charged CDs can cause bacterial cell death through electrostatic attraction to negatively charged cell membranes, potentially leading to the generation of active oxygen species.

This indicates that the antibacterial activity of synthetically produced CDs may largely depend on electrostatic interactions, contributing to their potential as antimicrobial agents in various applications.



**Figure 22:** Mechanism of antibacterial activity of CDs by electrostatic interaction

## CHAPTER 5

### CONCLUSIONS

#### 5.1 Conclusions

This work successfully synthesized CDs employing a hydrothermal method using *Lawsonia inermis* leaves extract, an approach that emphasizes sustainability and green chemistry principles. The hydrothermal synthesis route was validated by the observation of green fluorescence under UV light at 365 nm, signifying the formation of carbon dots. Comprehensive characterization techniques, including UV-visible spectroscopy, FTIR, XRD, and zeta potential measurement, corroborated the successful synthesis and provided insights into the structural and chemical nature of the CDs.

The UV-visible spectroscopy results showed a distinct absorption peak, attributable to the  $\pi-\pi^*$  transitions, indicating the quantum confinement effects and optical properties of CDs. FTIR analysis confirmed the presence of various functional groups such as amine, hydroxyl, carbonyl, and carboxyl, suggesting a rich surface chemistry conducive to biological interactions. The XRD patterns suggested an amorphous structure, a characteristic beneficial for certain applications due to its broad surface area and functional versatility. The zeta potential of the CDs was measured to be -29.3 mV, indicating a stable colloidal system that could be beneficial for biomedical applications.

The antibacterial activity of the synthesized CDs was evaluated against a spectrum of bacterial strains, including *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538P), *Klebsiella pneumoniae* (ATCC 700603) and fungal *C. albicans* (ATCC2091) through the agar well diffusion method. This activity could be attributed to the surface chemistry and quantum confinement effects of the CDs, highlighting their application in addressing antimicrobial resistance challenges.

In conclusion, this study shows the potential of *Lawsonia inermis* leaves extract as a viable and sustainable source for the synthesis of carbon dots and also presents the antibacterial properties of these nanomaterials. The research underscores the feasibility of utilizing plant extracts for the synthesis of carbon dots, offering a pathway toward the development of eco-friendly, cost-effective, and non-toxic materials for various

applications, including antimicrobial treatments. Future studies may explore the mechanistic aspects of the CDs' antibacterial action and expand their application horizon across different domains of science and technology.

## **5.2 Limitations**

- The study's characterization methods for CDs, though comprehensive, did not include advanced imaging techniques like SEM or TEM, limiting detailed morphological analysis.
- Antibacterial efficacy was assessed using agar well diffusion, which is a preliminary qualitative method. Quantitative analysis like MIC and MBC was not performed, which could provide a more nuanced understanding of the CDs' effectiveness.
- Mechanisms behind the antibacterial activity of CDs were hypothesized but not experimentally validated, leaving the exact interactions between CDs and bacterial cells unclear.

## **5.3 Recommendations for Future Work**

- Incorporate advanced microscopic techniques such as SEM and TEM to acquire detailed insights into the CDs' morphology and topography.
- Conduct quantitative antibacterial tests like MIC and MBC to establish the CDs' potency in comparison to standard antibiotics.
- Perform detailed mechanistic studies to understand the interaction between CDs and bacterial cells, potentially using techniques like flow cytometry and confocal microscopy.
- Explore broader applications of CDs in drug delivery, bioimaging, sensing, and photocatalysis, and investigate the functionalization of CDs for specific uses.
- Scale up the green synthesis process and assess its commercial viability for mass production of CDs.
- Conduct a comprehensive environmental impact assessment, including a lifecycle analysis of CDs, to ensure the sustainability of their production and application.

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## APPENDIX



Lawsonia Leaves



Powder form of sample leaves



Magnetic Stirring



Filtration



12 hours  
+Hydrothermal Device



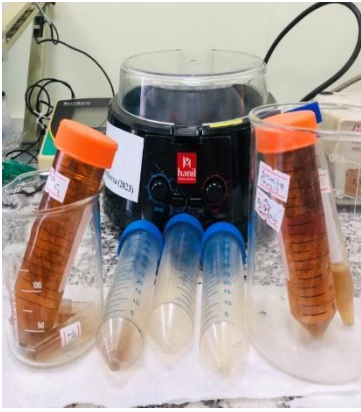
Filtration



Filtration Solution



Solution form of  
Carbon Dots



Centrifuge Device



Centrifuge Process



Solution after Centrifugation



Solution before Dialysis



Dialysis Process



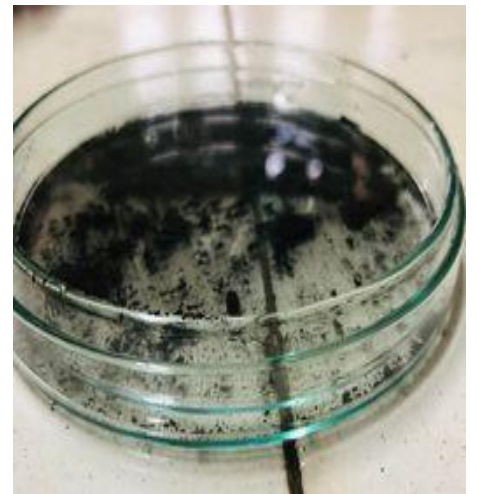
Solution After Dialysis



Freeze Dryer Device



Freeze Dryer Process



Powder form of Carbon Dots

## A. Phytochemical Screening Protocol

### 1. Tests for Alkaloids

For testing alkaloids, three different types of tests were carried out.

**Mayer's Test:** 1 mL of filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). The formation of a yellow-colored precipitate indicated the presence of alkaloids.

**Wagner's Test:** 1 mL of extract was mixed in a test tube with 3 drops of Wagner's reagent prepared beforehand. The formation of a reddish-brown precipitate showed the presence of alkaloids.

**Dragendorff's Test:** 2 mL of extract was taken in a test tube with 0.2 mL dilute HCl and 1 mL of Dragendorff's reagent and left for a few mins. A positive result is indicated by the presence of an orange-brown precipitate.

### 2. Test for Terpenoids

**Chloroform Test:** The plant extract was taken in a test tube with a few mL of chloroform, and added concentrated sulfuric acid carefully to form a layer and observed for the presence of reddish-brown color.

**Liebermann-Burchard Test:** 1 mL extracts were treated with chloroform, acetic anhydride, and drops of H<sub>2</sub>SO<sub>4</sub> and observed for the formation of a dark green color.

### 3. Test for Flavonoids

**Alkaline Reagent Test:** Extracts were treated with a few drops of sodium hydroxide solution. The formation of an intense yellow color, which became colorless with the addition of dilute acid, indicated the presence of flavonoids.

**Lead Acetate Test:** Extracts were treated with a few drops of lead acetate solution. The formation of a yellow color precipitate indicated the presence of flavonoids.

**Pew's Tests:** To 2-3 mL extract, zinc powder was added, followed by dropwise addition of conc. HCl. The formation of purple-red or cherry color indicated the presence of flavonoids.

**Shinoda Tests:** A few pieces of magnesium were mixed with 2-3 ml extract followed by dropwise addition of concentrate HCl and boiled for 5 minutes. The formation of a magenta color indicates the presence of flavonoids.

### 4. Test for steroid compounds

**Salkowaski's Test:** 1 mL plant extract was taken in a test tube and dissolved with 10 mL chloroform, and then an equal volume of concentrated sulphuric acid was added to the test tube by sides. The upper layer in the test tube turned red, and the sulphuric acid

layer showed a yellow color with green fluorescence to show the presence of steroids.

#### 5. Test for Quinones

To 2 mL of extract, a few drops of conc. H<sub>2</sub>SO<sub>4</sub> was added. Color formation indicated the presence of the quinoid compound.

#### 6. Tests for Saponins

Froth Test: Extracts were diluted with distilled water to 20mL, and this was shaken in a graduated cylinder for 15 minutes.

Foam Test: 0.5 g of the extract was shaken with 2 mL of water.

#### 7. Test for Tannins

FeCl<sub>3</sub> Test: 5 mL of extract solution was allowed to react with 1 mL of 5% ferric chloride solution. Greenish-black coloration indicated the presence of tannins.

Potassium Dichromate Test: 5 mL of the extract was treated with 1 mL of 10% aqueous potassium dichromate solution. The formation of a yellowish-brown precipitate suggested the presence of tannins.

Lead Acetate Test: 5 mL of each type of extract and a few drops of freshly prepared 1% lead acetate were dissolved together. Yellow precipitate showed a positive result.

#### 8. Test for Phenols

Ferric Chloride Test: Equal amounts of 1% ferric chloride solution and 1% potassium ferrocyanide were mixed. To 2mL extract, 3 drops of this freshly prepared mixture were added. The formation of a bluish-green or dark-green color was taken as positive.

Liebermann's Test: The extracts were heated with sodium nitrite, added H<sub>2</sub>SO<sub>4</sub> solution diluted with water, and an excess of dilute NaOH and observed for the formation of deep red, green, or blue color.

#### 9. Detection of Carbohydrates

Molisch's Test: Filtrates were treated with two drops of alcoholic  $\alpha$ -naphthol solution in a test tube. The formation of the violet ring at the junction indicated the presence of carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently.

Orange-red precipitate indicated the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolyzed with dilute HCl, neutralized with alkali, and heated with Fehling's A & B solutions. The formation of a red precipitate indicated the presence of reducing sugars.

#### Test for Proteins

The extract was diluted in 10 mL of distilled water and filtered with Whatman no. 1

filter paper. 2 mL of filtrate was heated with a few drops of Millon's reagent (The reagent is made by dissolving metallic mercury in nitric acid and diluting with water). A reddish-brown coloration or precipitate indicated the presence of tyrosine residue, which occurs in nearly all proteins.

Xanthoproteic Test: The extracts were treated with a few drops of conc. nitric acid. The formation of a yellow color indicates the presence of proteins.

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