

**GREEN SYNTHESIS AND CHARACTERIZATION OF  
CARBON DOTS USING *TINOSPORA CORDIFOLIA*  
STEM/LEAVES AND STUDY OF ITS ANTIBACTERIAL  
ACTIVITY**

**A DISSERTATION WORK SUBMITTED FOR  
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER  
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**SUBMITTED BY**

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INSTITUTE OF SCIENCE AND TECHNOLOGY  
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**JUNE, 2023**

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This dissertation entitled, “Green Synthesis and Characterization of Carbon Dots using *Tinospora cordifolia* Stem/leaves and Study of Its Antibacterial Activity” by Ms. Asmita Thapa Magar under the supervision of Asst. Prof. Dr. Deval Prasad Bhattarai, Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu, Nepal, and under co-supervision of Asst. Prof. Hari Bhakta Oli, Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu, Nepal, hereby submitted has been approved for partial fulfillment of the requirement for completion of her Master of Science (M.Sc.) Degree in Chemistry. This dissertation has not been submitted to any other university or institution previously for the award of a degree.

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## ABSTRACT

*Tinospora cordifolia* is a common medicinal plant, also known as Gurjo, and belongs to the Menispermaceae family. It has various medicinal applications including antibacterial, anticancer, antioxidant, digestive problems, anemia, and diarrhea. Carbon dots is a subgroup of quantum dots and zero-dimensional fluorescent nanomaterials with quasi-spherical morphology with a size of less than 10 nm. Being smaller and less toxic, Carbon dots are widely applicable in the treatment of infectious diseases and also in biosensors, bioimaging, gene delivery, catalysis, and drug delivery. Using *Tinospora cordifolia* stem/leaf aqueous extract, the carbon dots were synthesized by the green method in aqueous media. The synthesized Carbon dots showed deep green fluorescence under a UV lamp at 365 nm. The prepared Carbon dots were characterized by UV-visible spectroscopy, FTIR, and XRD to study their morphology. The zeta potential and band gap of synthesized Carbon dots were found to be -16.1 mV and 3.14 eV respectively, which revealed that the synthesized CDs is stable and semiconducting in nature. By agar well diffusion, the antibacterial activity of synthesized Carbon dots against *staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* was found to be 3 mm, 7 mm, and 6 mm respectively.

**Keywords:** *Tinospora cordifolia*, Carbon dots, green synthesis, Characterization, antibacterial activity

## LIST OF ACRONYMS AND ABBREVIATIONS

QDs	Quantum Dots
CDs	Carbon Dots
CQDs	Carbon Quantum Dots
QY	Quantum Yield
ROS	Reactive Oxygen Species
DNA/RNA	Deoxyribonucleic Acid/Ribonucleic Acid
FTIR	Fourier Transform Infrared Spectroscopy
FE-SEM	Field Emission Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
HRTEM	High Resolution Transmission Electron Microscopy
UV-Vis Spectroscopy	Ultraviolet Visible Spectroscopy
XRD	X-Ray Diffraction
XPS	X-ray Photoelectron Spectroscopy
EDX	Energy Dispersive X-ray Analysis
IR Spectroscopy	Infrared Spectroscopy
EDX/EDS	Energy Dispersive X-Ray Analysis
MTT	(3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl Tetrazolium bromide) tetrazolium reduction
DPPH	2,2-diphenyl-1-picrylhydrazyl
AFM	Atomic Force Microscopy
DLS	Dynamic Light Scattering
TGA	Thermo-Gravimetric Analyzer
FS	Fluorescence Spectroscopy
RS	Raman Spectroscopy
ATCC	American Type Culture Collection

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# CHAPTER 1

## INTRODUCTION

### 1.1 Background

Nanotechnology deals with the study of nanomaterials in a way for the innovation of industrial uses and rapid growth such as in the medicinal field for drug delivery systems, diagnostic biosensors, and imaging probes as well as advancements in manufacturing, packaging, durability, and bioavailability (Hulla, Sahu, and Hayes, 2015). Nanomaterials are materials with at least one dimension in the nanometer range (1-100 nm). These materials have unique physical and chemical properties such as catalytic activity, electrical and thermal conductivity, optical properties, and better morphology such as smaller size and larger surface area than the bulk materials. All these special properties makes them extensively exploited in various research areas such as antimicrobial, magnetic, electronic, catalytic, medication, water treatment, and photocatalysis (Baptista *et al.*, 2018; Nithya and Kalyanasundharam, 2019).

Nanoparticles, nano bars, nano cables, nanotubes, nanobelts, nanofibers, nanospheres, and quantum dots are all morphologically categorized nanomaterials. Structures based on carbon, such as fullerenes, polymers, and composite materials, fall into the first group, whereas metallic (Au, Ag, Cu, Al, Zn, Cd), semi-metallic (Se, Si), magnetic (Se, Si), and ceramic materials go into the second (Hernández-Díaz *et al.*, 2021).

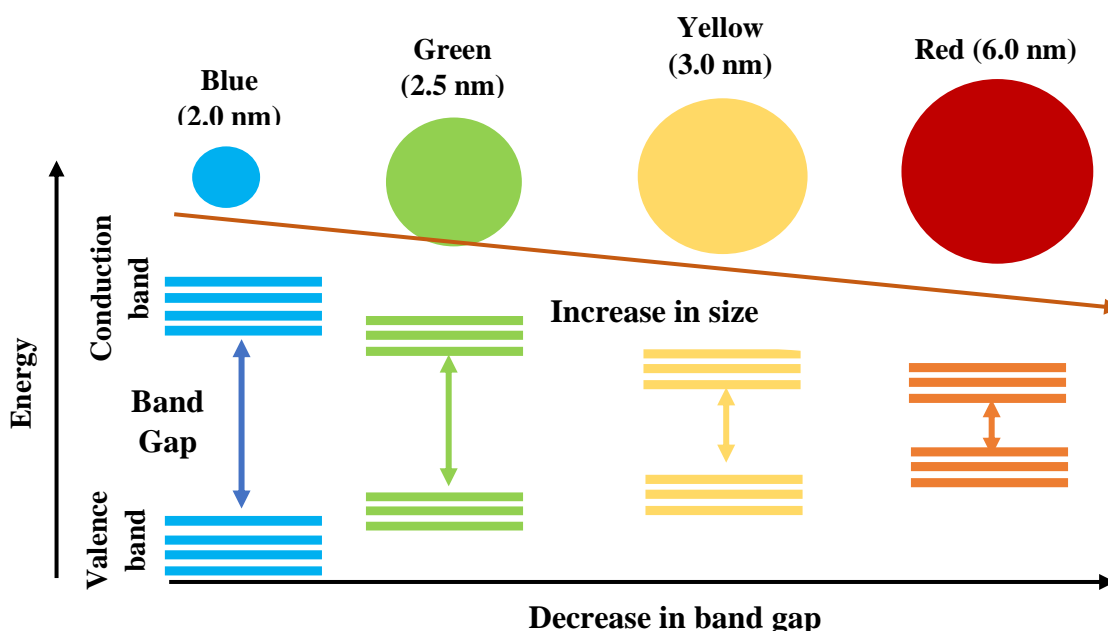
Nanomaterials can be divided into four categories based on dimension: zero-dimensional, one-dimensional, two-dimensional, and three-dimensional (Singh, Yadav, and Mishra, 2020). Quantum dots, hollow spheres, nano-lens, and other particles with all dimensions at the nanoscale belong to zero-dimensional nanomaterials (Jeevanandam *et al.*, 2018; Singh *et al.*, 2020).

Nanomaterials can be synthesized by physical, chemical, and biological methods. Compared to the physical and chemical methods, biological ways of nanomaterials synthesis using a microorganism, enzymes, fungus, and plant extract are environmentally friendly and less toxic (Hasan, 2015). The synthesis of nanomaterials using plant extracts is generally termed as green synthesis method. It is because extracts are environmentally friendly and benign reducing capping agents to stabilize the synthesized nanomaterials (Parveen, Banse, and Ledwani, 2016). Because of low toxicity, less expensive, and ease of characterization, studies have shown that green

approaches are more efficacious for the synthesis of nanomaterials (Gour and Jain, 2019).

## 1.2 Quantum Dots

Quantum dots (QDs) are particles with reduced physical dimensions (2-10 nm) and are semiconductor crystals of nanometer scale that are made of II-VI or III-V elements (Jamieson *et al.*, 2007; Wagner *et al.*, 2019). These are composed of a semiconductor core, a shell covering it, and a capping agent that improves solubility in an aqueous environment. Moreover, due to the quantum confinement effect, they show distinctive optical features such as size and composition variable light emission, wide absorption spectra, size dependent emission spectra, and highly fluorescence with high quantum yield (Reshma and Mohanan, 2019). In accordance with the band gap of the material, they absorb white light and then reemit a certain hue a few nanoseconds later (Valizadeh *et al.*, 2012). In quantum dots, the size and band gap are inversely correlated. According to fluorescence research, the electromagnetic spectrum shifts from red to blue when the quantum dot size decreases due to an increase in emission frequencies (Figure 1.1). This makes the quantum dot's excitation and emission variable (Karmakar, 2015).



**Figure 1.1:** A schematic diagram to show the correlation between size of CDs particle and band gap

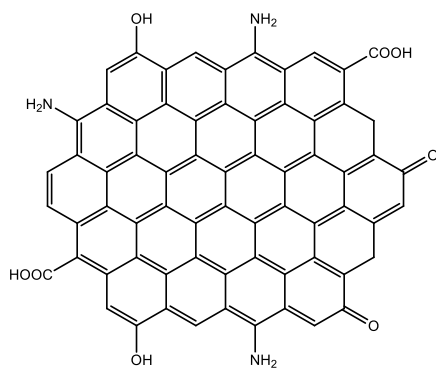
Quantum dots can be synthesized using top-down methods like ion beam lithography, electron beam lithography, and etching, as well as bottom-up methods like wet chemical synthesis, sol-gel synthesis, microemulsion synthesis, physical and chemical vapor deposition, and other hydrothermal and solvothermal methods, and green method using biomaterials (Karmakar, 2015).

The majority of the chemical and physical properties of QDs vary greatly from those of a basic semiconductor. QDs could be used in a variety of products, including solar cells, transistors, diode lasers, light-emitting diodes (LEDs), biomedical devices, inkjet printers, sewage treatment systems, spin-coating, and quantum computers (Mohamed *et al.*, 2021).

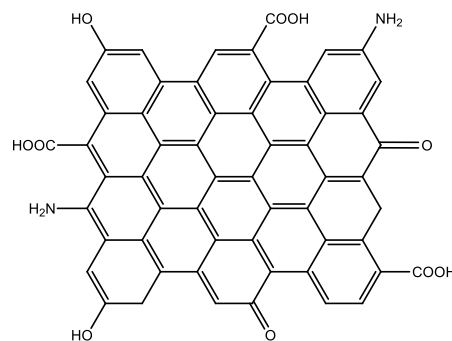
### 1.3 Carbon Dots

Carbon Dots (CDs) are recognized as a subgroup of QDs (Y. Liu *et al.*, 2020) and are a novel class of carbon-based zero-dimensional nanoparticles with a quasi-spherical morphology and a size of less than 10 nm. CDs are also known as carbon nanodots or fluorescent nanoparticles or carbon quantum dots. It exhibits a strong UV absorption peak and with good water solubility, it emits intense fluorescence when illuminated, has high biocompatibility, and is eco-friendly (Jamaludin, Rashid, and Tan, 2019; Kaiming *et al.*, 2021; Yao *et al.*, 2019).

Depending on the precursors and synthesis pathways used, CDs can have crystalline or amorphous morphologies. In addition, CD's, the shell layers are either filled with hydrogen atoms or functional groups like  $\text{NH}_2$ ,  $\text{OH}$ ,  $\text{COOH}$ , etc., while the core is a  $\text{sp}^2$  hybridized carbon and its edges may be arranged in a zigzag or armchair pattern (Figure 1.2) (John, Nair, and Vinod, 2021).



**Zig-Zag edged Carbon dots**



**Armchair edged Carbon dots**

**Figure 1.2:** Zig-zag and Armchair pattern of carbon dots

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

#### **1.3.1.1 Absorption properties**

CDs often exhibit optical absorption with a tail that reaches into the visible spectrum in the UV region (Wang and Hu, 2014). The absorption bands are associated with a  $\pi$ - $\pi^*$  transition of aromatic  $sp^2$  carbon's C=C bonds and an n- $\pi^*$  transition of the carbon core's C=O bond, or they may have developed via a surface state transition involving electron lone pairs (Liu, 2020).

#### **1.3.1.2 Fluorescence properties**

The excitation wavelength ( $\lambda_{ex}$ ) dependence of emission and intensity was responsible for the fluorescence of CDs and it could be a result of the optical choice of variously sized nanoparticles (quantum effect) or various emissive traps on the surface of CDs (X. Wang *et al.*, 2019; Wang and Hu, 2014). The primary fluorescence origins of CDs at this time are surface state, quantum confinement effect, conjugated structure, self-trap excitons, edge defect, free zigzag sites, and multi-emissive centers (M. Liu, 2020; Tuerhong *et al.*, 2017). And the temperature, pH, and concentration all have a significant impact on the fluorescence of CDs (Liu, 2020). The quantum yield (QY), which is the ratio of photons emitted to photons absorbed by the CDs, can be used to express the fluorescence intensity. This can be done using a comparative approach with standard samples that have been thoroughly characterized and whose QY values are known (Kumar, Kumar, and Gedanken, 2020).

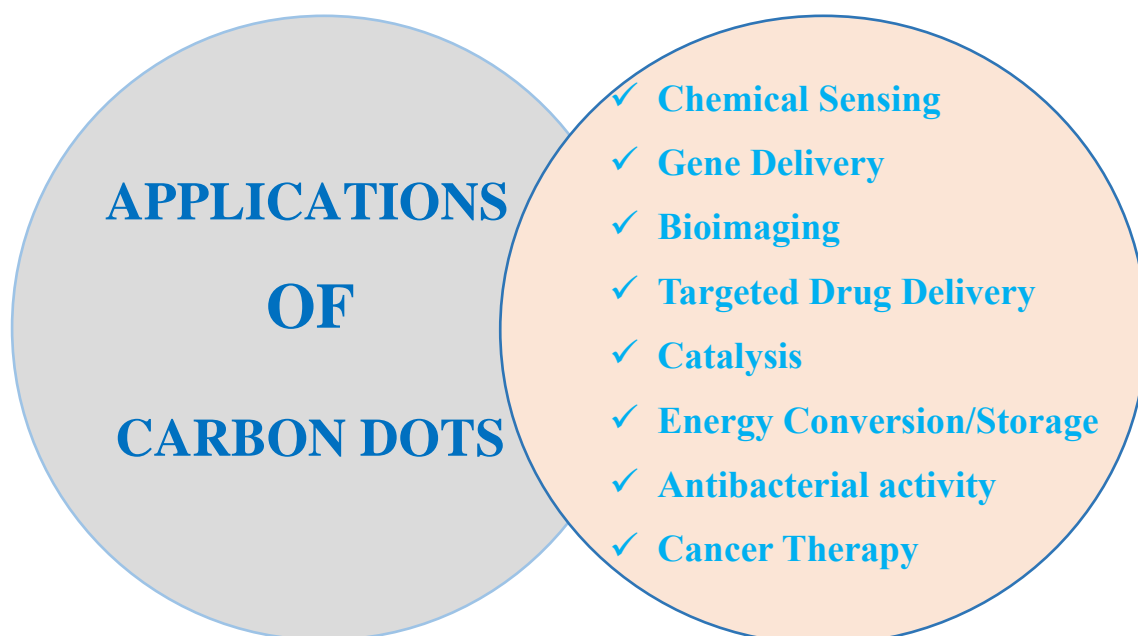
#### **1.3.1.3 Electrochemical properties**

CDs exhibit great performance for electrochemical processes because of their special electron-transfer properties and a sizable specific surface area (Tian *et al.*, 2021; Zou *et al.*, 2019). Electrocatalytic performance is enhanced by the presence of several surface functional groups (-OH, -NH<sub>2</sub>, etc.), strong intra-molecular contacts, and doped hetero-atoms (N, P, S, B, etc.) that effectively alter the electronic characteristics of carbon atoms by intra-molecular charge transfer (Tian *et al.*, 2021). Also, they are widely available and cost-effective.

### **1.3.2 Applications of Carbon Dots**

Full carbon nanomaterials, which are composed entirely of carbon, are less toxic than other nanomaterials. Additionally, CDs have a smaller particle size, making them easier to enter living cells. This gives CDs a wide range of potential biological applications

(Zuo *et al.*, 2015). And are also gaining traction as potential candidate systems for photocatalysis, energy conversion/storage, Chemical sensing , and biomedical applications such as biosensors, bioimaging, gene delivery, catalysis, and drug delivery platforms for cancer, neurodegenerative diseases, and infectious diseases (Tejwan, Saha, and Das, 2020; Thulasinathan *et al.*, 2020).



**Figure 1.3:** Application areas of carbon dots

Carbon dots outperform metal-based quantum dots (QDs) in terms of nature of water solubility, facile functionalization, chemical inertness, reduced perniciousness, acceptable biocompatibility, and fluorescent qualities, resulting in a wide range of applications, including biomedical applications (Zaib *et al.*, 2021a). And also cost-effective, and less toxic than other quantum dots (Ying Lim, Shen, and Gao, 2015).

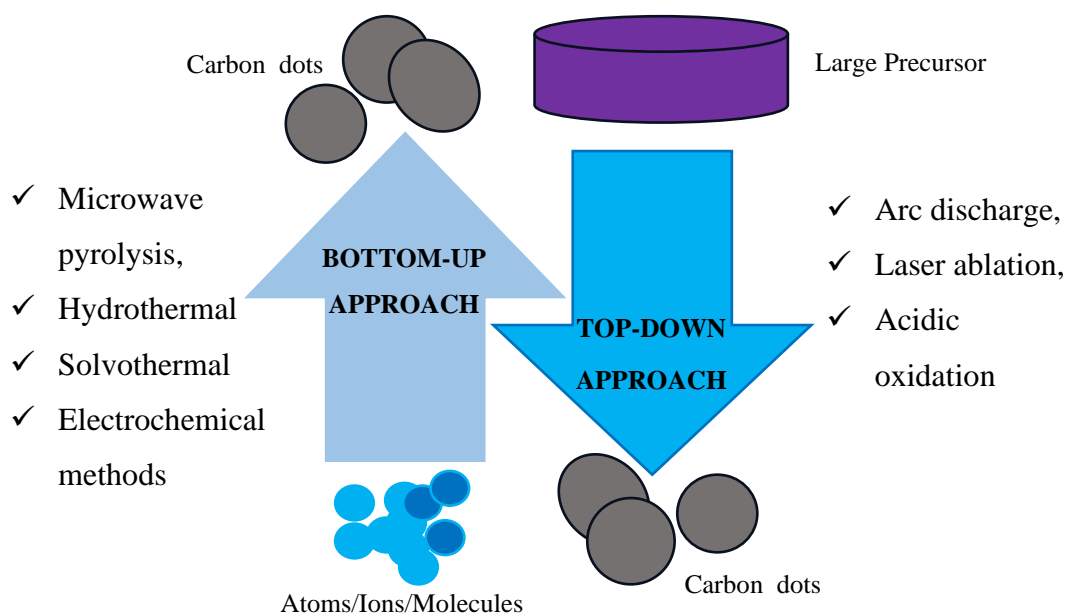
### **1.3.3 Antibacterial Activity of Carbon Dots**

Due to their tiny size, surface charge, amphiphilic affinity, and surface functional groups from beginning materials, CDs can pierce and light up bacterial cells. Furthermore, CDs can be functionalized for microbial identification by coating them in antibiotics or fusing them with other nanoparticles like hydrogels and magnetic nanoparticles. (Li *et al.* 2022; Lin, Bao, and Wu 2019). The larger production of reactive oxygen species than bacterium's antioxidant defense, biofilm growth inhibition, maintenance of antibiotic features, and mainly cell wall damage are included under the antibacterial activity of Carbon dots (Wu *et al.*, 2021). When carbon dots

attached to the surface of bacteria, induction of reactive oxygen species (ROS) production in response to light, breaking down and entering the bacterial cell wall/membrane, initiation of oxidative stress with DNA/RNA damages, resulting in modifications to or suppression of crucial gene expressions, and the development of oxidative damage to intracellular biomolecules including proteins are all part of the mechanism of action (Dong *et al.*, 2020).

#### 1.4 Synthesis of Carbon Dots

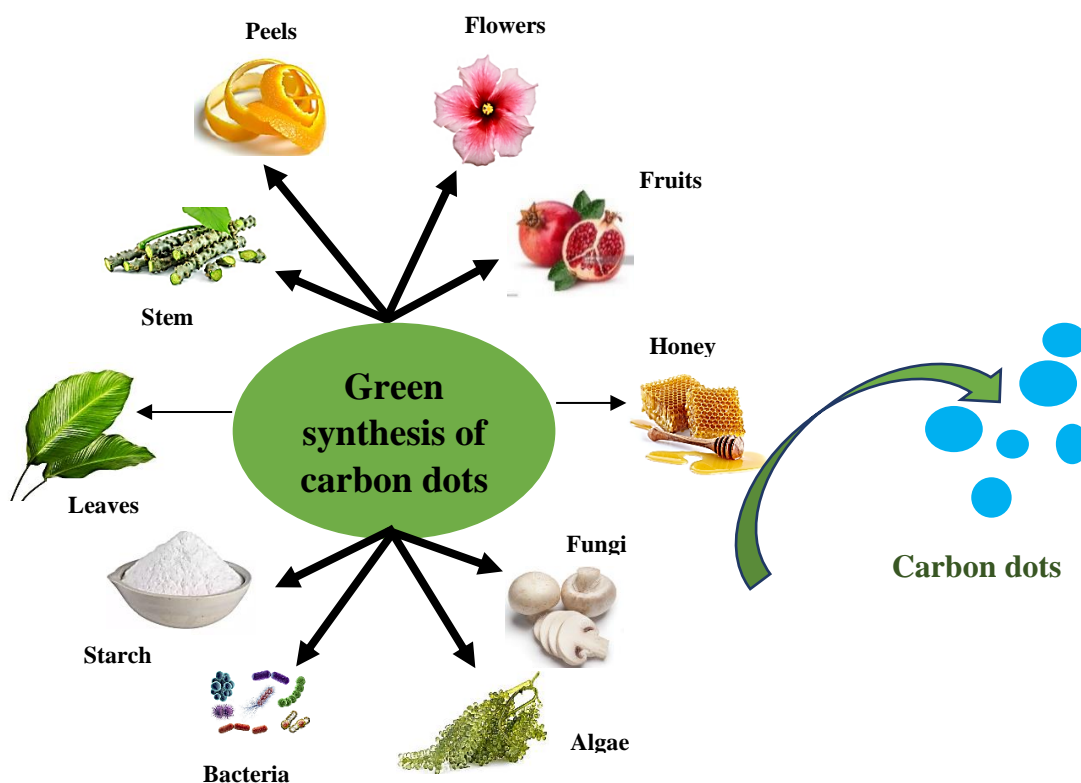
The top-down approach like arc discharge, laser ablation, acidic oxidation, and bottom-up approach such as combustion routes, microwave pyrolysis, hydrothermal/solvothermal, and electrochemical methods are included as common methods for the synthesis of the carbon dots (X. Wang *et al.*, 2019). However, the usage of costly energy-consuming instruments, for example, is a major disadvantage of laser ablation and ion beam radiation. Chemical oxidation of a suitable carbon source is far from being a green process when a strong acid is used. So, cost-effective, clean, benign, and approachable green approach to the synthesis of CDs is always a good choice (Ramanan *et al.*, 2016).



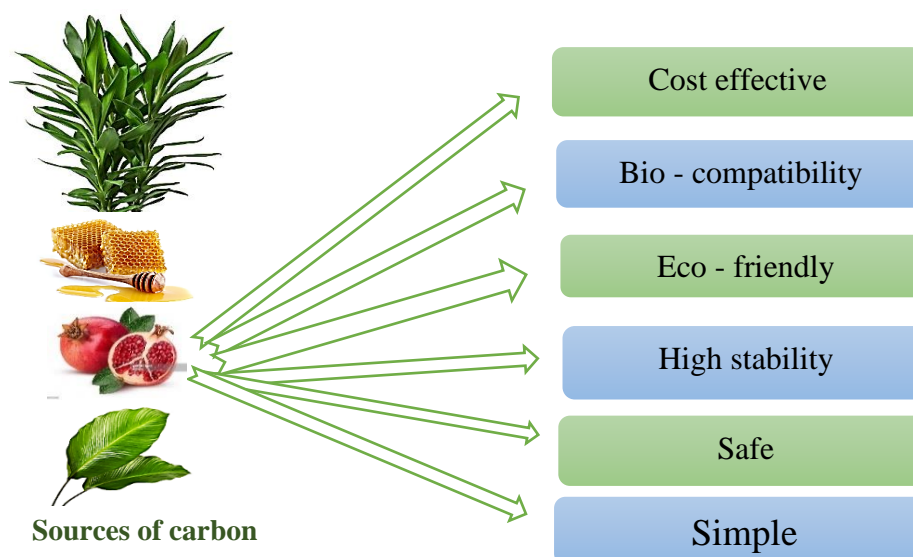
**Figure 1.4:** Carbon dots preparation method (Top-down and Bottom-up)

The best way to synthesize CDs is always to use a greener method (Thulasinathan *et al.*, 2020). The utilization of natural, sustainable carbon sources in the green synthesis of CDs is a particularly appealing research issue. As a result, natural green sources for

CDs have been recorded as grape juice, lime juice, milk, potato, orange juice, plant leaves, oats, and waste biomass (Sachdev and Gopinath, 2015).



**Figure 1.5:** Sources of carbon for synthesis of carbon dots



**Figure 1.6:** Advantages of the green synthesis method

### 1.5 *Tinospora cordifolia* as Carbon Sources

*Tinospora cordifolia* is a common medicinal herb in the area of natural medicine and Ayurveda (Basalingappa, 2017) and also known as "Guduchi" in Sanskrit, is a genetically varied, big, deciduous climbing bush with greenish-yellow characteristic blooms that grow at higher altitudes and belongs to the Menispermaceae family (Saha and Ghosh, 2012).

*Tinospora cordifolia* has several constituents that have been isolated. Alkaloids, diterpenoid lactones, steroids, glycosides, aliphatic chemicals, and polysaccharides are among them (Spandana *et al.*, 2013). Furthermore, the stems of *Tinospora cordifolia* included cellulose in amounts of 23.02% and hemicellulose in amounts of 3.70%, according to proximate analysis (Mahima *et al.*, 2014).

#### **Taxonomic classification of *Tinospora cordifolia***

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Ranunculales

Family: Menispermaceae

Genus: *Tinospora*

Species: *T.cordifolia*



**Figure 1.7:** *Tinospora cordifolia*

This plant's stem is succulent, with lengthy, filamentous, plump and thick, and climbing tendencies. The thin, paper-like outer bark is brown to grayish in color. In general, leaves have a bright green color, but as they age, they turn a yellowish green to yellow color. This plant's leaves are Plain, alternative, long-petioled (15 cm), globular, pulvinate, heart-shaped, and distorted in half and middle as shown in Figure 1.7 (Bharathi *et al.*, 2018).



**Figure 1.8:** *Tinospora cordifolia* stem

The *Tinospora cordifolia* is commonly employed in indigenous medical practices. Additionally, It is also used for the treatment of general weakness, digestive problems, loss of appetite, fever in youngsters, diarrhea, gonorrhea, urinary infections, viral hepatitis, and anemia. The current study reports on the scientific examination of *Tinospora cordifolia*'s medical effectiveness as an antibacterial, antioxidant, and anticancer substance (Mishra, Kumar, and Pandey, 2013). The chemical components of the plant, such as diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds, essential oils, a combination of fatty acids, and polysaccharides, are responsible for the plant's medicinal effect (Srivastava and Singh, 2021). Not only medicinal value but also these constituents are the potential source of carbon dots.

## **1.6 Objectives of the Study**

### **1.6.1 General Objective**

The main objective of this research is to carry out the green synthesis and characterization of Carbon dots and their antibacterial effect using *Tinospora cordifolia* leaf/stem extract.

### **1.6.2 Specific Objective**

The specific objectives of the study can be outlined as follow;

- Synthesis of carbon dots using *Tinospora cordifolia* stem/leaf extract by green synthesis method.
- Characterization of the green synthesized Carbon dots by using UV-vis spectroscopy, XRD, FTIR.
- Study of the zeta potential and band gap of synthesized carbon dots.
- Study of the antibacterial activity of synthesized Carbon dots against Gram-positive and Gram-negative bacteria by agar well diffusion method.

## CHAPTER 2

### LITERATURE REVIEW

Several researches have been carried out on synthesized carbon dots and their biomedical applications including antibacterial activity using different carbon sources. Some of them are summarized here:

Thulasinathan *et al.* synthesized fluorescent green/blue CDs using oyster mushrooms by hydrothermal method and characterized them by UV-vis spectroscopy, FTIR, TEM/HRTEM, and FE-SEM. The antibacterial activity was examined by the agar well diffusion method against *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. The result showed the maximum zone of inhibition (25mm) related to the highest antibacterial activity was observed with 100  $\mu\text{L}$  of CDs against *P. aeruginosa* and also observed the anticancer activity against cancer cells with a half maximum inhibitory concentration  $\text{IC}_{50}$  of 3.34  $\mu\text{g}/\text{mL}$  and able to detect  $\text{Pb}^{2+}$  ion (Thulasinathan *et al.*, 2020).

Using ampicillin as a precursor by hydrothermal method, Gao *et al.* synthesized the CDs, and characterization was carried out by TEM, UV-vis spectroscopy, XPS, SEM, and FTIR and observed the high quantum yield (23%). The results revealed that the CDs exhibit strong bacterial activity against *S.aureus* and *L. monocytogenes* under visible light illumination at low concentrations (Gao *et al.*, 2020).

Li *et al.* synthesized CDs from vitamin 'C' using an electrochemical one-step process, and then examined them using TEM, HRTEM, XPS, UV-vis spectroscopy, and FTIR. Additionally, the agar well diffusion method was used to study antibacterial activity against Gram +ve and Gram -ve pathogens at various concentrations, and the concentration at 100 $\mu\text{g}/\text{mL}$  showed outstanding antibacterial capabilities and also observed the antifungal activity against *Rhizoctonia solani* and *Pyricularia grisea* and found effective at 300  $\mu\text{g}/\text{mL}$  concentration (Li *et al.*, 2018).

Muktha *et al.* investigated the antimicrobial and cytotoxic properties of CDs produced by the microwave technique from pomegranate and watermelon peels and characterized them by UV-vis spectroscopy, FTIR, HRTEM, fluorescence spectroscopy, and Raman spectroscopy. Agar well diffusion was used to study the antibacterial action, whereas the food poison technique was used to study the antifungal activity. In comparison to

the positive control ciprofloxacin at 10  $\mu\text{g/mL}$  ( $19.67 \pm 0.40$ ), the CDs synthesized from pomegranate peel extract (P-CDs) and watermelon peel extract (W-CDs) demonstrated anti-bacterial action at a concentration of 80  $\mu\text{L}$  ( $19.67 \pm 0.37$ ). When compared to fluconazole, which has an antifungal effect ( $85 \pm 0.90$ ), P-CDs ( $61.00 \pm 1.90$ ) have much more antifungal activity, while W-CDs had no effect. Additionally, powerful anti-cancer effects for cell lines have been demonstrated by P-CDs and W-CDs (Muktha *et al.*, 2020).

Gao *et al.* studied the synthesis of fluorescent CDs by hydrothermal method using beer yeast powder and characterization by FTIR, XPS, UV-vis spectroscopy, and SEM. The CDs were prepared with a quantum yield of 21% and released multicolor fluorescence when activated at various wavelengths. And by agar well diffusion, the highest antibacterial activity was observed against *Escherichia coli* with increasing concentration and within 120 min of exposure to visible light (Gao *et al.*, 2019).

Pandiyan *et al.* carried out the synthesis of carbon quantum dots (CQDs) from sugarcane industrial waste by hydrothermal technique. The synthesized CQDs were characterized by XRD, XPS, FTIR, HRTEM, and UV- visible spectroscopy. And carried out the fluorescence analysis and observed the quantum yield of 17.98%. By well diffusion method, good antibacterial activity was found against all tested *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Vibrio cholera*, and *Pseudomonas aeruginosa* and also proved to be a good source for optical switching applications (Pandiyan *et al.*, 2020).

Saravanan *et al.* synthesized the multifunctional carbon dots using turmeric leaves (*Curcuma longa*) by hydrothermal method. The characterization was done by TEM, UV- visible spectroscopy, Fluorescence spectroscopy, FTIR, XPS, and ROS generation was observed on a Bruker X-band spectrometer using 5'-dimethyl-1-pyrroline-N-oxide). Intense fluorescence with bright blue color at  $\lambda_{\text{ex}}/\lambda_{\text{em}} = 362/429$  nm was observed by fluorescence spectra. By colony forming unit method, both *E. coli* and *S. aureus* were found to be destroyed at low concentrations compared to *K. pneumoniae* and *S. epidermis* (Saravanan *et al.*, 2021).

Liu *et al.* studied the synthesis of fluorescent carbon dots from carrot juice by hydrothermal method for in vitro cellular imaging. The characterization was carried out by TEM, XRD, FTIR, XPS, UV-visible spectroscopy, and fluorescence spectrometry.

The quantum yield was found to be as high as 5.16 %. Cytotoxicity was examined by an MTT assay using human HaCaT cells. Then, a cell imaging experiment was carried out using CDs cultured human epidermal keratinocytes (HaCaT) cells under a confocal microscope and observed the distinct multicolor images (Y. Liu *et al.*, 2017).

Sachdev and Gopinath synthesized the CDs from coriander leaves by hydrothermal method and characterized them by UV- visible, fluorescence spectroscopy, TEM, SEM, XRD, and FTIR. For antioxidant activity, a DPPH assay was applied and observed the increase in scavenging activity with an increase in CD concentration. The fluorescence base sensor was used for ion sensing and showed that the Fe<sup>3+</sup> caused the strongest fluorescence quenching effect on CDs resulting in higher selectivity towards Fe<sup>3+</sup> ion. Cytotoxicity was studied by MTT assay and also bioimaging was carried out (Sachdev and Gopinath, 2015).

M. Wang *et al.* studied the application of CDs in Fe<sup>3+</sup> and Ascorbic acid detection, and cell imaging. The CDs were synthesized using *Osmanthus fragrans* Lour by hydrothermal method and characterized by UV-visible spectroscopy, fluorescence spectrometer, TEM, XRD, FTIR, XPS, and EDS. Cytotoxicity was measured by MTT assay and observed low toxicity, and used for cell imaging. The result showed higher selectivity for Fe<sup>3+</sup> ion and was also able to detect the ascorbic acid (M. Wang *et al.*, 2019).

Ghann *et al.* synthesized the CDs by hydrothermal treatment of sodium citrate and urea and characterized them by FTIR, EDS, TEM, UV-vis spectroscopy, electrochemical impedance spectroscopy, and fluorescence lifetime measurements. It was used as a sensitizing agent for the fabrication of dye-sensitized solar cells and the result showed the strong capability of CDs to be used as a sensitizing agent (Ghann *et al.*, 2019).

Joshi, Mathias, and Mishra synthesized the CDs by hydrothermal method from coconut leaves and characterized them by FTIR, UV-vis spectroscopy, TEM, and XRD. It was studied as a biosensor matrix in the bioimaging of cancer cells and the DNA hybridization sensor for pancreatic cancer. And by the well diffusion method, CDs were discovered to be antibacterial inhibitors against *B. subtilis*, *M. smegmatis*, and *S. aureus* (Joshi, Mathias, and Mishra, 2018).

Using polyethyleneimine and citric acid as a source by hydrothermal method, Demirci *et al.* prepared the nitrogen-doped CDs and characterized them by FTIR, TEM, XRD,

Dynamic light scattering, UV-vis spectroscopy, and fluorescence spectroscopy. The antibacterial activity was found strong against the Gram -ve bacteria compared to Gram +ve bacteria. And CDs were able to penetrate and passed through the dermal tissue and suggesting their capability to administer topical drugs (Demirci *et al.*, 2020).

Using plant *Andrographis paniculata*, Naik *et al.* prepared the blue fluorescent carbon dots by hydrothermal method with a quantum yield of 15.10% and characterized by fluorescence spectrophotometer and Eppendorf bio-spectrometer, XRD, FTIR, TEM, EDX, and thermogravimetric analysis. The observations by MTT assay showed that in Gram +ve and Gram -ve clinically isolated strains of bacteria, carbon dots exhibited negligible cytotoxicity, and were capable of cellular imaging of human breast cancer cells and also observed the free radical scavenging properties by DPPH assay (Naik *et al.*, 2020).

Atchudan *et al.* prepared the nitrogen-doped carbon dots using peach juice by hydrothermal method and characterized them by XRD, FTIR, XPS, FESEM, EDX, and HRTEM. And the result revealed that the prepared nitrogen-doped carbon dot showed bright fluorescence with excellent biocompatibility with *Candida Albicans* cells and was employed for live cell imaging as the fluorescent probe (Atchudan *et al.*, 2018).

W. Liu *et al.* produced the nitrogen-doped CDs (N-CDs) utilizing rose-heart radish by hydrothermal method with a fluorescent quantum yield of 13.6% and characterizations by TEM and HRTEM, FTIR, UV-vis spectroscopy, XPS, and fluorescence spectrophotometer. MTT assay was applied for cell cytotoxicity where Human uterine cervical squamous cell carcinoma (SiHa) cells were exposed to doses of 0.0 mg/mL to 1.0 mg/mL for 24 h. Even when N-CDs were in high concentrations (1.0 mg/mL), the survival rate was better than 85%. And the results showed that the N-CDs exhibit negligible cell toxicity. The bright-field images of the SiHa cells cultured with N-CDs by laser scanning confocal microscopic shows the morphology of cells and fluorescence was found upon addition of  $\text{Fe}^{3+}$  ion shows high sensitivity to the  $\text{Fe}^{3+}$  ion (W. Liu *et al.*, 2017).

Bandi *et al.* green synthesized the luminous carbon dots from onions using a simple autoclave and investigated their morphology using TEM, XPS, FTIR, DLS, XRD, TGA, Raman spectroscopy, and fluorescence spectroscopy. The study of metal ions shows that the presence of  $\text{Fe}^{3+}$  ions dramatically reduces the fluorescence intensity of

CDs, making them highly selective fluorescent probes for Fe<sup>3+</sup> ions. Additionally, even at concentrations of 1 mg/mL, the viability of both normal and cancer cells is still 90%, demonstrating that these CDs are less toxic and highly biocompatible for multicolor cell imaging (Bandi *et al.*, 2016).

Green synthesis of carbon dots was studied by Zaib *et al.* using the leaves of *Elettaria cardamomum* (EC) and was characterized by XRD, FTIR, Raman, UV-vis, and photoluminescence spectroscopy. According to the findings, congo red dye degraded most rapidly in acidic conditions at pH 4 and a dye concentration of 5 ppm for 50 min. In an alkaline medium with a pH of 8 and a 5 ppm dye concentration over 55 minutes, the best degradation of the methylene blue dye was found (Zaib *et al.*, 2021).

Gedda *et al.* prepared the carbon dots using the autoclaving process from prawn shells, and they noted a 9 % quantum yield and blue fluorescence at 365 nm under a UV lamp. By using UV-vis spectroscopy, FTIR, TEM, XRD, EDX, and fluorescence spectrophotometer, the morphology was investigated. And with a small detection limit of 5 nM, the carbon dots demonstrated outstanding selectivity and sensitivity toward Cu<sup>2+</sup> (Gedda *et al.*, 2016).

Roy *et al.* synthesized the Carbon dots using turmeric by hydrothermal method. The prepared CD's size was found to be in the range of 10 nm by FETEM and under UV light, the CD displayed a distinct color change from yellowish-brown to green. In comparison to Gram-negative bacteria (*E. coli*), the CDs had greater antibacterial activity against Gram-positive bacteria (*L. monocytogenes*). And also showed strong antioxidant activity in the DPPH assay. The prepared CDs were discovered to have a zeta potential of  $-17.2 \pm 1.2$  mV (Roy *et al.*, 2022).

**Table No. 2.1:** The outline of the literature review of synthesis, characterization, and application of synthesized carbon dots using various carbon sources;

<b>CARBON SOURCES</b>	<b>SYNTHETIC METHOD</b>	<b>CHARACTERIZATION</b>	<b>APPLICATIONS</b>	<b>REFERENCE</b>
Oyster Mushroom	Hydrothermal	UV-vis, FTIR, TEM/HRTEM FE-SEM,	Antibacterial activity, anticancer activity	Thulasinathan <i>et al.</i> , 2020
Ampicillin	Hydrothermal	TEM, UV-vis, XPS, SEM, FTIR	Antibacterial activity	Gao <i>et al.</i> 2020
Pomegranate and Watermelon peels	Microwave	FTIR, HRTEM, FS, Raman spectroscopy, UV-vis	Antimicrobial activity and cytotoxicity	Muktha <i>et al.</i> 2020
Vitamin 'C'	One-step Electro- Chemical	TEM, HRTEM, XPS, UV-vis, FTIR	Antibacterial activity, Antifungal activity	Li <i>et al.</i> 2018
Beer yeast powder	Hydrothermal	FTIR, XPS, UV- vis, SEM	Antibacterial activity	Gao <i>et al.</i> 2019
Sugarcane industrial waste	Hydrothermal	XRD, XPS, FTIR, UV-vis, HRTEM	Antibacterial activity	Pandiyan <i>et al.</i> 2020
Turmeric leaves	Hydrothermal	TEM, UV-vis, Fluorescence Spectroscopy (FS), FTIR, XPS	Antibacterial activity	Saravanan <i>et al.</i> 2021
Carrot juice	Hydrothermal	TEM, XRD, FTIR, XPS, UV- vis, FS	Cytotoxicity and cell imaging	Liu <i>et al.</i> , 2017
Coriander leaves	Hydrothermal	UV-vis, FS, TEM, FTIR, SEM, XRD, FTIR	Antioxidant activity, Ion sensing, Cytotoxicity and Bioimaging	Sachdev and Gopinath 2015

<i>Osmanthus fragrans</i>	Hydrothermal	UV-vis, FS, TEM, XRD, FTIR, XPS, EDS	Cytotoxicity and cell imaging, Fe <sup>3+</sup> and Ascorbic acid detection	M. Wang <i>et al.</i> 2019
Sodium citrate and Urea	Hydrothermal	FTIR, EDS, TEM, UV-vis, Electrochemic-al impedance spectroscopy, Fluorescence lifetime measurements	As a sensitizing agent for fabrication of dye sensitized solar cell.	Ghann <i>et al.</i> 2019
Coconut leaves	Hydrothermal	FTIR, UV-vis, TEM, and XRD	Biosensing and antibacterial activity	Joshi <i>et al.</i> 2018
Polyethyleneimine and carrot juice	Hydrothermal	FTIR, TEM, XRD, Dynamic lightscattering, UV-vis, FS	Antibacterial activity, Skin permeability	Demirci <i>et al.</i> 2020
<i>Andrographis Paniculata</i>	Hydrothermal	FS and Eppendorf spectrometer, FTIR, XRD, TEM, EDX, TGA	Cytotoxicity and Cell imaging, Antioxidant activity	Naik <i>et al.</i> 2020
Peach juice	Hydrothermal	XRD, FTIR, XPS, FESEM, EDX, HRTEM	Bioimaging	Atchudan <i>et al.</i> 2018
Rose-heart radish	Hydrothermal	TEM and HRTEM, FTIR, UV-vis, XPS and FS	Fe <sup>3+</sup> detection and cell imaging	W. Liu <i>et al.</i> 2017
Onions	Autoclaving method	TEM, XPS, FTIR, DLS, XRD, TGA, Raman spectroscopy (RS), and FS	Fe <sup>3+</sup> detection and multicolor cell imaging	Bandi <i>et al.</i> 2016
<i>Elettaria cardamomum</i>	Ultrasonicatio-n method	XRD, FTIR, RS,UV-vis,	As a photocatalyst in dye degradation	Zaib <i>et al.</i> 2021

		photoluminescence spectroscopy		
Prawn shells	Autoclaving method	UV-vis, FTIR, FS, EDX, XRD, TEM	Cu <sup>2+</sup> detection	Gedda <i>et al.</i> 2016
Turmeric	Hydrothermal	FETEM, XPS, XRD, FTIR	Zeta potential, antibacterial, antioxidant	Roy <i>et al.</i> 2022

As can be seen in Table 2.1, numerous studies on carbon dots have been conducted using biological materials like plants and fruits as well as chemical compounds. These materials were then synthesized using hydrothermal, electrochemical, microwave, and ultrasonication processes, and then they were characterized using UV-visible spectroscopy, FTIR, EDX, XRD, FESEM, TEM, AFM, FS, Raman spectroscopy, TGA, and XPS for their morphological observation. The synthesized carbon dots were examined for several applications, including metal detection, ion sensing, bioimaging, antibacterial, anticancer, and antifungal.

Furthermore, the chemical substances were used for the study of carbon dots in previous studies which are relatively harmful to some extent. To make it more biocompatible and less toxic, the study will be carried out by synthesizing carbon dots only using water. Additionally, the antibacterial activity was previously reported in numerous studies, but the mechanisms were not thoroughly addressed. Therefore, the mechanism behind the antibacterial action of carbon dots against microorganisms will be covered.

## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1 Materials Collection

*Tinospora cordifolia* (Gurjo) stem and leaves were collected from Chhebetar, Gorkha, Nepal (Longitude 84.57061, latitude 28.00160). A Google map of the sample collecting site is given in Figure 9.



**Figure 3.1:** Google map of sample collecting site indicating the location

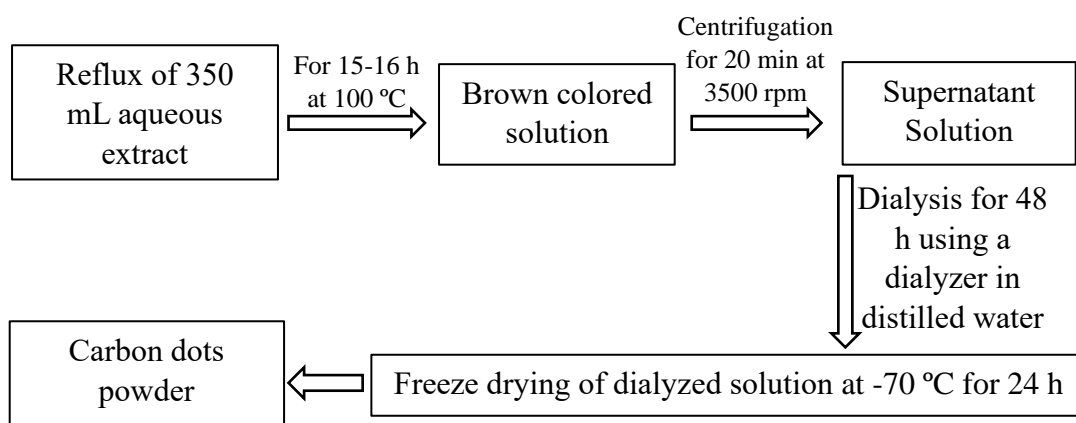
#### 3.2 Preparation of Extract

The cold maceration method was employed for the preparation of an aqueous extract of carbon dots. For this, the collected Gurjo stem and leaves were shade dried for one month and the dried sample (stem/leaves) was ground using a grinder to make powder. Then, 50 g of powdered sample (25 g stem + 25 g leaves) was soaked in 450 mL distilled water and divided into conical flasks, and shaken in a rotary flask shaker for 24 h at 160 rpm. After that, the mixed sample was filtered using a muslin cloth, and the filtrate was collected and stored in a refrigerator at 4 °C for further use.

#### 3.3 Green Synthesis of Carbon Dots

Carbon dots were synthesized by the green method for which 350 mL of prepared extract was taken into a 500 mL round bottom flask and refluxed for 15-16 h at 100 °C

until the solution turned brown. Then, the brown-colored solution was centrifuged at 3500 rpm for 20 min to separate the large impurities/particles and the supernatant solution was collected. For purification, the collected supernatant solution was dialyzed for 48 h using a dialyzer with MWCO of 12,000 Da in distilled water. Every 24 hours, the distilled water was changed twice. After dialysis, the obtained purified solution was taken under a UV lamp at 365 nm to observe its fluorescence. Then, the solution was freeze-dried at  $-70\text{ }^{\circ}\text{C}$  for 24 h to make carbon dots in solid form and the prepared powdered CDs were taken in a vial for further use.



**Figure 3.2:** Flow sheet diagram of green synthesis of carbon dots

### 3.4 Characterization of Synthesized Carbon dots

For the morphological study of Carbon dots, UV-visible spectroscopy, Fluorescence Transmission Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD) were used. In addition, Zeta potential and band gap measurements were also carried out.

#### 3.4.1 UV visible measurement

Synthesized carbon dots were characterized by UV-visible spectroscopy for which an aqueous solution of carbon dots was used to record spectra. 5 ppm solution of carbon dots was used to record spectra in the range 200-900 nm with a scan interval of 5 nm. Measurement was carried out at the Department of Chemistry, Amrit Campus, Kathmandu using double-beam UV-visible spectrometer (Labtronics, LT-2802).

#### 3.4.2 FTIR measurement

FTIR characterization was also employed for the characterization of carbon dots using Perkin Elmer 10.6.2 spectrometer at the Department of Chemistry, Amrit Campus, Kathmandu, Nepal. A dry sample was used to record the spectra in ATR mode in the range  $450\text{-}4000\text{ cm}^{-1}$  with a scanning interval of  $4\text{ cm}^{-1}$ .

### **3.4.3 X-ray Diffraction**

X-ray diffraction measurement was carried out for the study of phase of synthesized carbon dots. Solid carbon dots sample was used to record the spectra. XRD measurement was carried out at Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bangalore, India.

### **3.4.4 Measurement of Zeta Potential**

0.01 g of synthesized Carbon dots was dissolved in 40 mL deionized water in a capillary cell and measurement was carried out at 25 °C using the Horiba instrument at Nepal Academy of Science and Technology (NAST), Lalitpur, Nepal.

### **3.4.5 Band Gap Measurement**

The band gap measurement of a synthesized carbon dots was carried out at Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bangalore, India.

## **3.5 Antibacterial Activity**

The antibacterial activity of carbon dots was studied by the agar well diffusion method at the Himalaya Research Institute of Biotechnology, Srijananagar, Bhaktapur.

### **3.5.1 Bacterial Culture for Antibacterial Activity**

The Gram +ve bacteria; *Staphylococcus aureus* (ATCC 6538P), *Bacillus subtilis* (ATCC 6051), and Gram -ve bacteria; *Escherichia coli* (ATCC 8739) were cultured using 5 mg of nutrient broth in 1 mL distilled water which was sterilized by autoclaving at 121 °C for 15 min and incubated for 24 h at 37 °C for bacterial growth.

### **3.5.2 Preparation of Agar Medium**

9 g of agar-agar was dissolved in 245 mL of distilled water in a conical flask. Then, it was sterilized by autoclaving at 121 °C for 1 h. After sterilization, the agar solution was taken into a different Petri dish and left until it gets solidified.

### **3.5.3 Antibacterial Test**

100 µL of each cultured bacterial solution was spread in each agar medium. Then, 5 µL of both CDs sample (5 µg/mL concentration) and Kanamycin as a positive control (5 µg/mL concentration) were taken into created wells in each petri dish agar medium and incubated for 24 h at 37 °C. After that, the zone of inhibition was observed and measured in each sample.

## CHAPTER 4

### RESULTS AND DISCUSSION

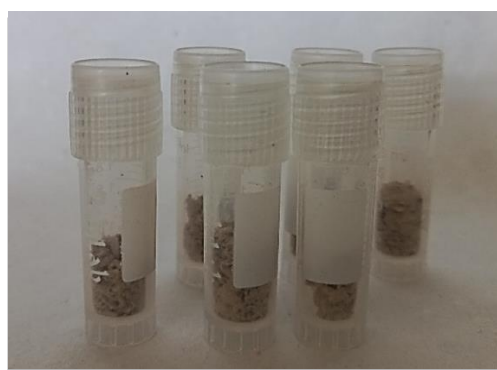
#### 4.1. Synthesis of Carbon Dots by Green Method

0.558 g of powdered carbon dots were prepared from 50 g of powder sample. Synthesized carbon dots in refluxing solution were freeze-dried and collected in vials for further study.

The purified brown-colored carbon dots solution showed deep green fluorescence under a UV lamp at 365 nm in accordance with (Mewada *et al.*, 2013) as shown in Figure 4.1(C-D). Green fluorescence results from the  $n-\pi^*$  transition's edge state. When electron-hole pairs from the carbon core recombine in a radiative manner, short-wavelength fluorescence (below 400 nm) results. However, because CDs have various oxygen-containing groups on their surface, the long-wavelength fluorescence (above 400 nm) is a result of the excited state's radiative relaxation into the ground state (M. Liu, 2020). Carbon dot fluorescence is caused by variations in particle size because of the quantum confinement effect since size is crucial for adjusting the band gap of CDs. Its fluorescence is also influenced by the amount of nitrogen, the level of surface oxidation, and the surface functional group (M. Liu, 2020; Tejwan *et al.*, 2020).

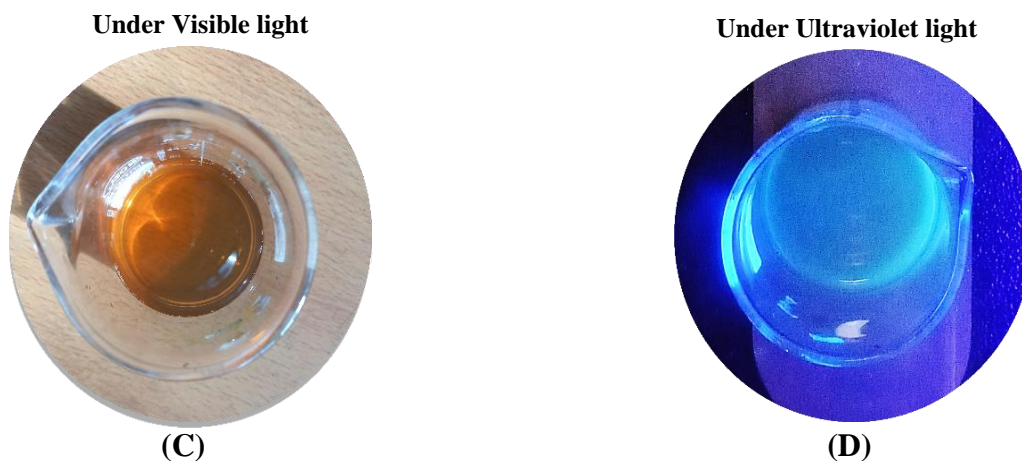


(A)



(B)

**Figure 4.1:** (A) Powdered CDs (B) Powdered carbon dots collected in vials

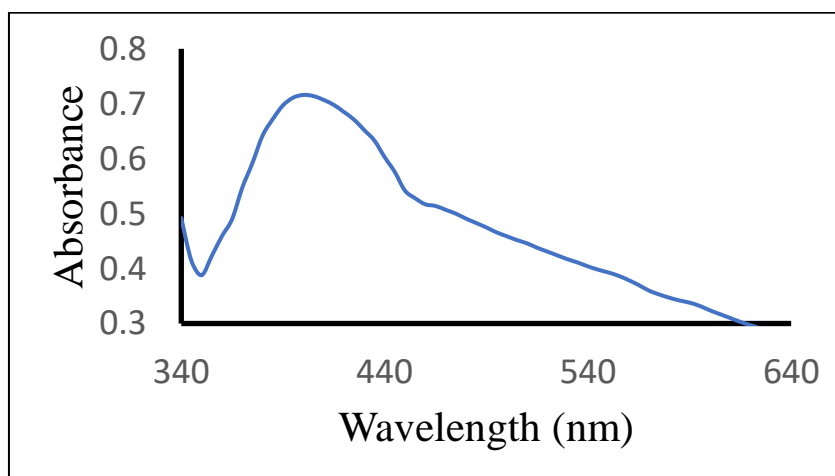


**Figure 4.2:** (C) Carbon dots under visible light and (D) Deep green fluorescence under ultraviolet light at 365 nm

## 4.2 Characterizations of Synthesized Carbon Dots

### 4.2.1 UV-visible Spectroscopy

UV-visible spectroscopy is used to determine the optical properties of Carbon dots. A cheap, straightforward, adaptable, non-destructive analytical technique suitable for a large class of organic chemicals, as well as some inorganic compounds is UV-vis spectroscopy. It calculates the wavelength-dependent absorbance or transmittance of light moving through a material (Rocha *et al.* 2018). The size of  $\pi$ -conjugated domains, fluctuation in the oxygen/nitrogen composition in carbon cores, and types and amounts of surface groups have the greatest effects on the absorption characteristics of CDs (J. Liu *et al.*, 2020).



**Figure 4.3:** UV-Visible spectrum of synthesized Carbon dots using *Tinospora cordifolia* stem/leaves

CDs generally exhibit optical absorption with a tail that reaches into the visible spectrum in the UV region (Y. Wang & Hu, 2014). The synthesized CDs showed broad absorption band at 400 nm in the UV-visible spectrum which is assigned to the  $n-\pi^*$  transition of the C=O of carbonyl group present in Carbon dots in respect to (Kumari *et al.*, 2022; J. Liu, Li, and Yang, 2020). The presence of carbonyl functional group at the surface of CDs was confirmed by FTIR of synthetic CDs. The  $n-\pi^*$  transition occurs when an electron in non-bonding (n) orbital of oxygen atom is excited to the antibonding ( $\pi^*$ ) orbital of carbon atom. It involves less energy compared to other electronic transition and gives absorption band at longer wavelength of ultraviolet region than other.

Furthermore, O-containing functional groups on CDs' surfaces, such as hydroxyl, carboxyl, and epoxy, might reduce the energy levels, leading to longer wavelength of ultraviolet region (M. Liu, 2020). In addition, the quantum confinement phenomenon also responsible for their absorption in UV region. The particle is small in size which causes the energy difference in between valence band and conduction band. The electronic transition occurs with high energy and shorter wavelength which is in ultraviolet region.

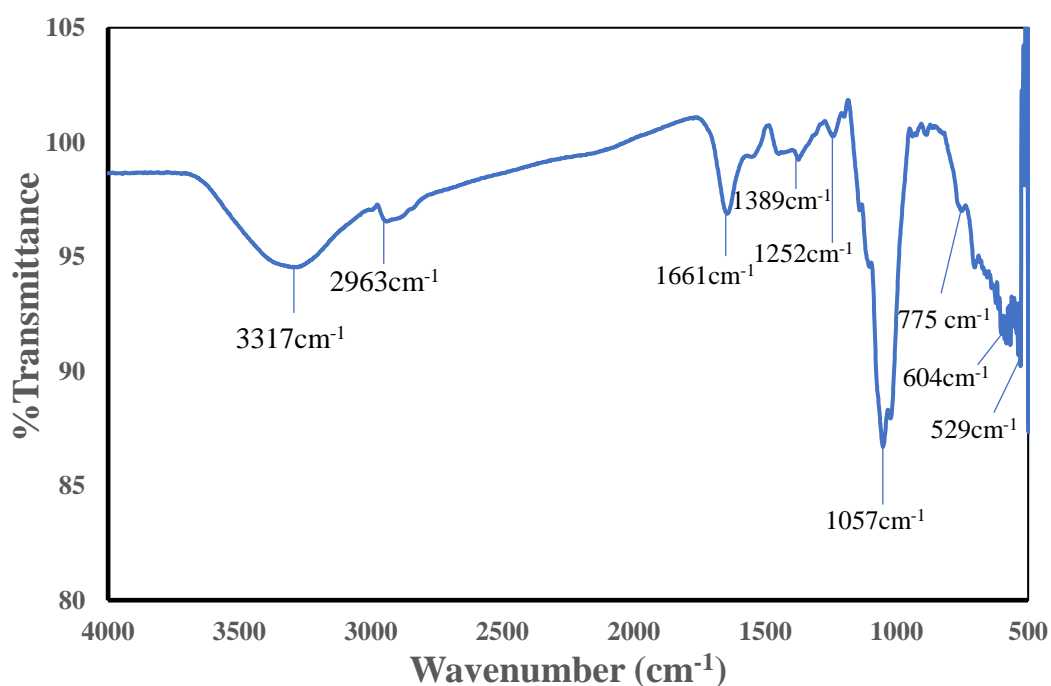
#### **4.2.2 Fourier Transform Infrared Microscopy (FTIR)**

One of the most effective methods for the identification of functional group is FTIR spectroscopy. Determination of the individual chemical components might be accomplished by understanding the wave number positions of the IR absorption bands in the spectrum (e.g., aromatic, amides) and it can be used for both qualitative and quantitative examinations (Mohamed *et al.*, 2017).

The FTIR spectrum of green synthesized Carbon dots from *Tinospora cordifolia* is shown in Fig 4.4. The stretching vibration of the –OH and –NH groups is responsible for the broad peak at  $3317\text{ cm}^{-1}$  (Song *et al.*, 2018). The peak at  $2963\text{ cm}^{-1}$  is attributed to C-H stretching, which may result from the presence of methyl or methylene groups linked to the aliphatic hydrocarbons in the extract of *Tinospora cordifolia* stem/leaf (Mewada *et al.*, 2013). The peak at  $1252\text{ cm}^{-1}$  is attributed to –OH bending vibration (Y. Liu *et al.*, 2017). C=O stretching vibration is attributed to the band at  $1661\text{ cm}^{-1}$

(Thulasinathan *et al.*, 2020; M. Wang *et al.*, 2019). The small peak at  $1389\text{ cm}^{-1}$  is for the stretching vibration of C–N (Saravanan *et al.*, 2021).

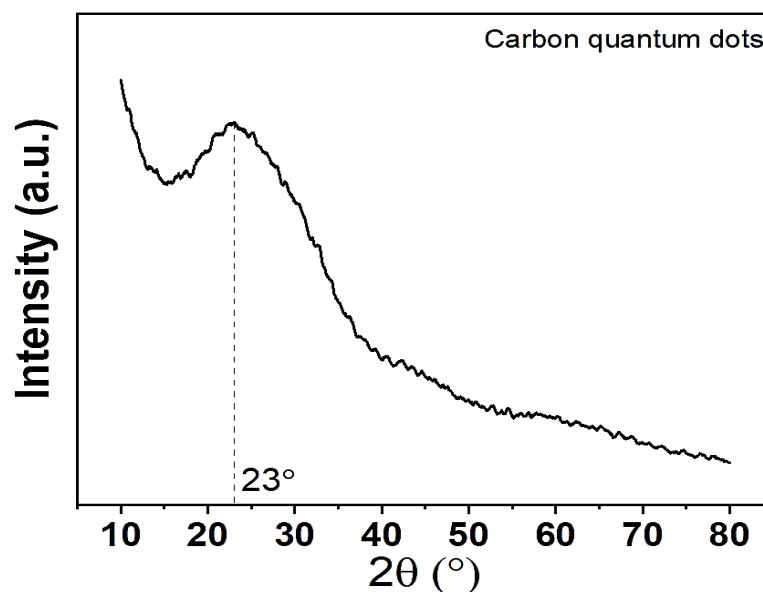
The strong peak at  $1057\text{ cm}^{-1}$  represents the stretching and bending vibrations of C–O in carboxyl groups (Sachdev & Gopinath, 2015). The flavonoid moieties were assigned to peaks between  $400$  and  $800\text{ cm}^{-1}$  (Thulasinathan *et al.*, 2020). According to findings and literature values, synthesized Carbon dots contain several amine, hydroxyl, carbonyl, and carboxyl functional groups.



**Figure 4.4:** Fourier Transform Infrared spectrum of green synthesized Carbon dots using *Tinospora cordifolia* stem/leaf

#### 4.2.3 X-ray Diffraction Spectroscopy (XRD)

In order to precisely examine the structure of crystalline phases, XRD techniques rely on crystals ability to diffract X-rays in a distinctive way. But because crystalline structures are periodic, they generate either constructive or destructive scattered radiation, which results specific diffraction patterns that may be examined to determine the crystal structure of various materials (Epp, 2016).



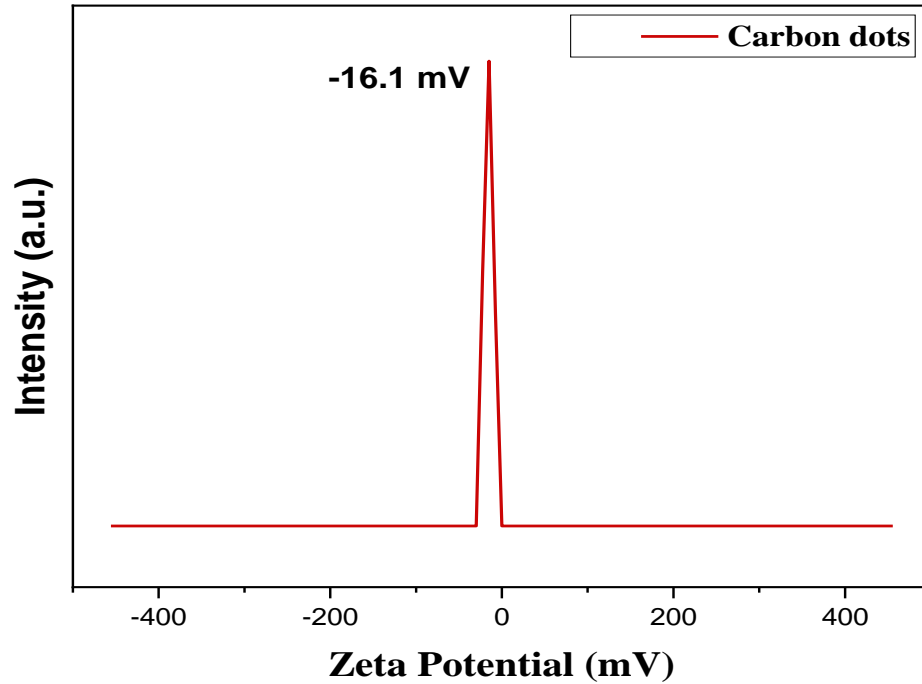
**Figure 4.5:** XRD spectrum of synthesized carbon dots using *Tinospora cordifolia* stem/leaves

The synthesized CDs' X-ray diffraction spectra revealed the presence of a significant peak at  $2\theta = 23^\circ$ , which belongs to the carbon dots' (002) diffraction plane. A wide peak at  $23^\circ$  reveals the carbonaceous core of CDs (Bandi *et al.*, 2016; Kumari *et al.*, 2022). The large peak with the (002) diffraction plane reveals the disorganized pattern of Carbon dots (Pandiyani *et al.*, 2020). This suggests that the green synthetic carbon dots are amorphous and have a weak crystalline structure (Zaib *et al.*, 2021).

#### 4.2.4 Zeta Potential Measurement

Zeta potential depends on the characteristics of both the nanoparticle and the medium and is determined by electrophoretic mobility measurements. The behavior, and toxicity of nanomaterials in environmental and biological systems can be studied using zeta-potential measurements (Lowry *et al.*, 2016). To assess Carbon dot's stability in solution, measurements of zeta potential is carried out (Roy *et al.*, 2022).

The zeta potential of synthesized Carbon dots was determined to be -16.1 mV, which was found to be close to the zeta potential measured by Roy *et al.* This is caused by the surface charge obtained from the breakdown of some functional groups present in carbon dots (Roy *et al.*, 2022). The negative zeta potential value of CDs revealed the presence of more negative charges at the surface of synthesized CDs. In addition, the repulsive force between negatively charged particles prevent agglomeration and maintain the stability of synthesized CDs.



**Figure 4.6:** Zeta potential of synthesized carbon dots

#### 4.2.5 Band Gap Measurement

A renowned and extensively researched quantum confinement phenomenon is semiconductor quantum dots' size-dependent band gap (Baskoutas and Terzis, 2006). The bandgap of the semiconductor grows because of the energy gap between the valence band and the conduction band (Singh, Goyal, and Devlal, 2018). In semiconductors, the band gap ' $E_g$ ' exists between the filled valence band and vacant conduction band in the range of  $\sim 0.5$  to  $\sim 3.5$  eV (Murphy and Coffey, 2002).

The synthesized Carbon dots from *Tinospora cordifolia* stem/leaf showed a band gap with 3.14 eV as shown in Figure 4.7 in accordance with (Murphy and Coffey, 2002). The energy of the band gap between the filled state and empty states grows as the particle size decreases due to the confinement of electrons and holes (Singh *et al.*, 2018). The observed band gap support the semiconducting nature of synthetic CDs as its value was found to be comparable with semiconductor.

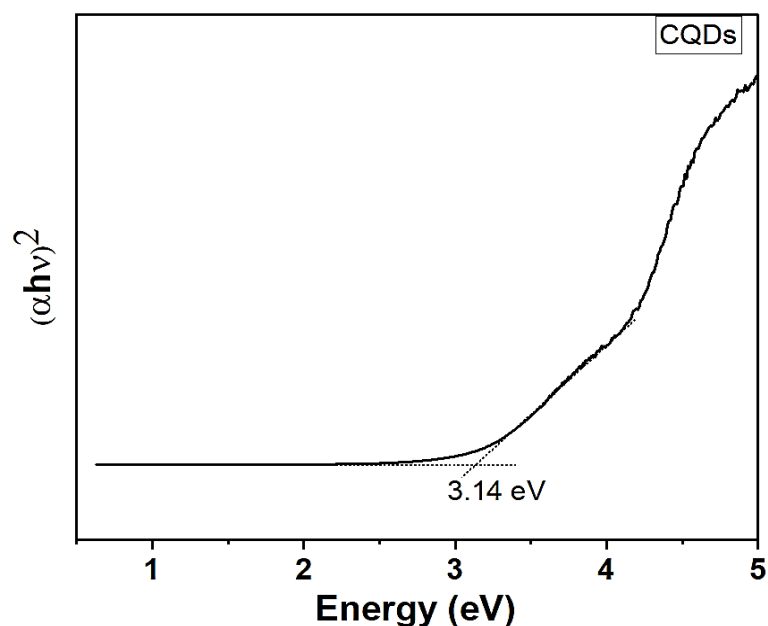


Figure 4.7: Band gap measurement of carbon dots using Tauc plot

### 4.3. Antibacterial Activity

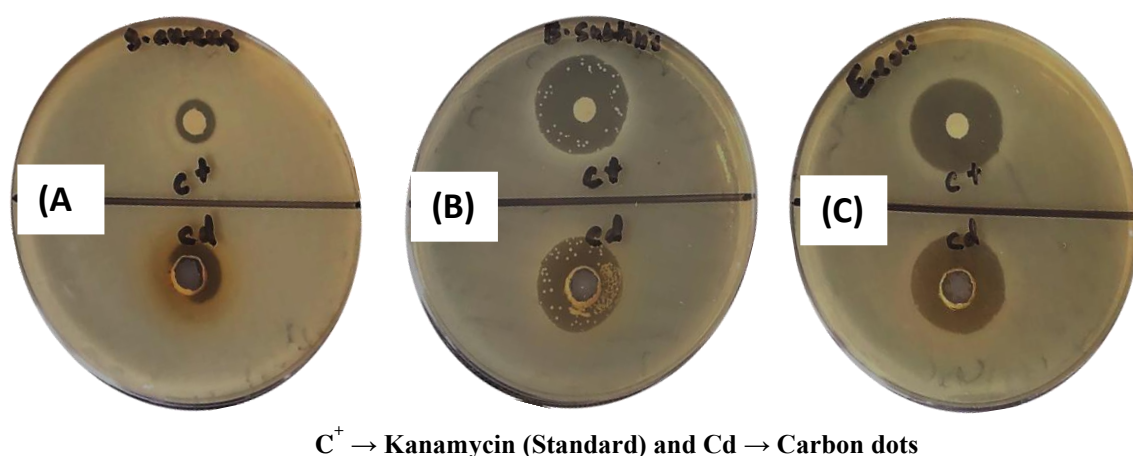
Antibacterial activity of synthesized Carbon dots was examined against two Grams +ve bacteria *Staphylococcus aureus* (ATCC 6538P) and *Bacillus subtilis* (ATCC 6051) as well as one Gram -ve bacteria *Escherichia coli* (ATCC 8739) by agar well diffusion method. The zone of inhibition observed for both Gram +ve and Gram -ve bacteria are shown in Table 4.1. Kanamycin was used as a positive control to compare with the obtained results from synthesized Carbon dots to observe their antibacterial activity.

Table 4.1: Antibacterial activity of synthesized Carbon dots by agar well diffusion

Pathogens	Concentration ( $\mu\text{g/mL}$ )	Zone of Inhibition (mm)	Positive Control Kanamycin (mm)
<i>Staphylococcus aureus</i> (ATCC 6538P) (Gram +ve)	5	3	5
<i>Bacillus subtilis</i> (ATCC 6051) (Gram +ve)	5	7	10
<i>Escherichia coli</i> (ATCC 8739) (Gram -ve)	5	6	10

The synthesized Carbon dots showed the potential antibacterial activity against *Bacillus subtilis* and *Escherichia coli* with a zone of inhibition of 7 mm and 6 mm respectively and are comparable to the standard positive control *Kanamycin* (10 mm zone of inhibition). However, the antibacterial activity against *Staphylococcus aureus* was found to be less with a zone of inhibition of 3 mm compared to standard positive control *Kanamycin* (5mm zone of inhibition). The zone of inhibition shown by carbon dots against bacteria and control are shown in Figure 4.8.

Because they sabotage secondary DNA/RNA structures, hinder bacterial walls during the diffusive entrance, and reduce vital gene expressions, the produced CDs efficiently stop *E. coli*, *B. subtilis*, and *S. aureus* from developing (B. Wang *et al.*, 2021). Several studies have shown that CDs may easily enter bacterial cells and disrupt cellular metabolism due to their small size, which is assumed to be the cause of their antibacterial activity (Roy *et al.*, 2022).



**Figure 4.8:** Antibacterial activity of synthesized Carbon dots from *Tinospora cordifolia* stem/leaf extract against (A) *S.aureus* (B) *B. subtilis* (C) *E.coli*

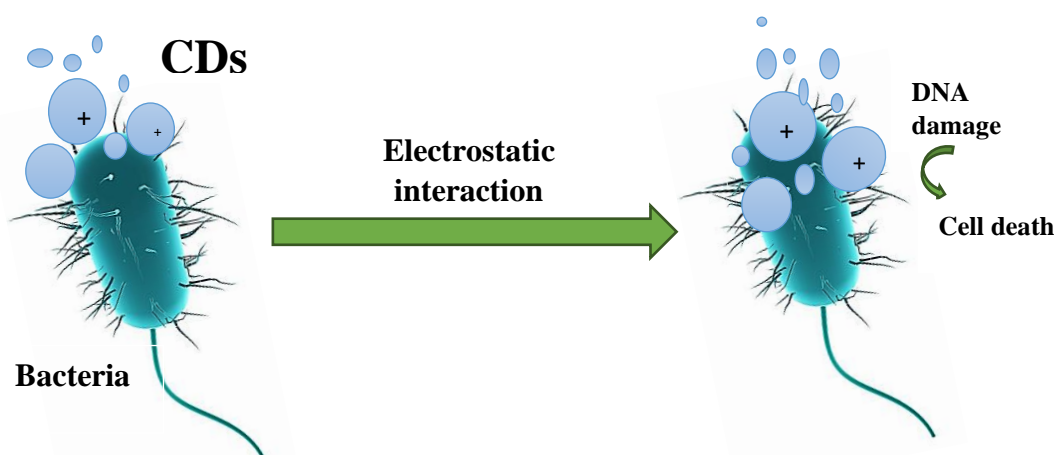
#### 4.3.1 Mechanism of Antibacterial Activity of Synthesized Carbon Dots

According to the literature, it has been generally hypothesized that the antibacterial mechanism of CQDs is based either on electrostatic interactions or ROS, or light irradiation (Pandiyani *et al.*, 2020). Carbon dots' antibacterial action is primarily attributed to the oxidative stress that is brought on by the ROS they produce (Wu *et al.*, 2021), associated with surface charge (Bing *et al.*, 2016), and also involves the damaging of cell walls or cell membranes (Li *et al.* 2018).

According to the negative value of the zeta potential measurement, the synthetic CDs have a more negative charge on their surface like hydroxyl groups, amino groups as revealed by FTIR spectrum. Additionally, Gram positive bacteria were the ones against which the synthesized CDs' antibacterial effectiveness was strongest. Therefore, it may be concluded that the mechanism of antibacterial activity of synthesized CDs is based on electrostatic interaction which is discussed as follow;

Due to electrostatic attraction, CDs can adhere to the surface of bacteria. CDs can penetrate the bacteria after adsorption because of their nano-size. CDs can destabilize DNA's double helix structure, which ultimately prevents bacterial growth (Song *et al.* 2018). Furthermore, the interactions between CDs and the cell membranes of bacteria are greatly impacted by the charge characteristics of CDs. Different bacterial reactions, such as apoptosis and death, can be brought on by negatively charged CDs (Wang *et al.*, 2021).

The highest antibacterial activity of CDs was observed against Gram positive bacteria *Staphylococcus aureus* (ATCC 6538P), *Bacillus subtilis* (ATCC 6051). This was due to electrostatic interaction between more negatively charge CDs surface and positively charged bacterial cell membrane which results the DNA/RNA damage, oxidative stress, damage of intracellular biomolecules including protein and eventually bacterial cell death.



**Figure 4.9:** Mechanism of antibacterial activity of CDs by electrostatic interaction

In addition, the synthesized CDs also showed antibacterial activity against Gram negative bacteria *Escherichia coli* (ATCC 8739) which might be due to the electrostatic interactions between a negatively charged bacterial cell membrane and positively

charged hydrogen atom on the CDs surface or the protonated forms of the nitrogen functional groups, which which most likely led to the production of active oxygen species, oxidative stress, and cell death.

Because of more negative charge on synthesized CDs surface than positive charge there is a strong electrostatic interaction between negatively charge CDs surface and positive bacterial cell membrane compared to interaction between positively charge CDs surface and negative bacterial cell membrane. Which leads to the greater antibacterial effect against Gram positive bacteria than Gram negative bacteria.

## CHAPTER 5

### CONCLUSION

Carbon dots have been synthesized successfully by a green method using *Tinospora cordifolia* stem/leaves extract. The formation of carbon dots was monitored by observing green fluorescence under a UV lamp at 365 nm. Results obtained from UV-visible spectroscopy, FTIR, XRD, zeta potential, and band gap measurement supported the formation of carbon dots. The maximum absorption peak at 400 nm in the UV-visible spectrum due to  $n-\pi^*$  transition, and absorbance peak due to the presence of different functional group amine, hydroxyl, carbonyl, and carboxyl functional groups reflected from FTIR spectra indicate the presence of the surface functional group in CDs. XRD pattern further support and suggest the amorphous structure of CDs. The zeta potential of synthesized CDs was found to be -16.1 mV, which was found to be quite stable. The band gap of synthesized CDs was found to be 3.14 eV, which was one of the reason to confirm the semiconducting nature of CDs. The antibacterial activity of CDs measured by agar well diffusion method suggested the good antibacterial activity of synthesized CDs against *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538P).

The findings from the synthesized CDs using the *Tinospora cordifolia* stem/leaves aqueous extract of *Tinospora cordifolia* lead to the following conclusion:

- The synthesized CDs was found to be highly fluorescent, amorphous, and semiconducting in nature along with broad absorption band and functional groups. So, it can be applicable for biological imaging, sensing, lighting, catalysis, energy storage, photovoltaics, optoelectronics, display technology, and drug delivery.
- It is possible to employ the synthesized CDs as a novel source for antibacterial applications because they exhibited good antibacterial activity.
- The Carbon dots can be prepared from plants using only water could be a tremendous advantage for future convenient, eco-friendly, cost-effective, and non-toxic research.

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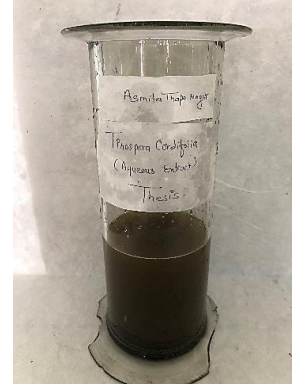
## APPENDIX



*Tinospora cordifolia*  
stem/leaf



*Tinospora cordifolia*  
stem/leaf powder



Aqueous solution of  
powder



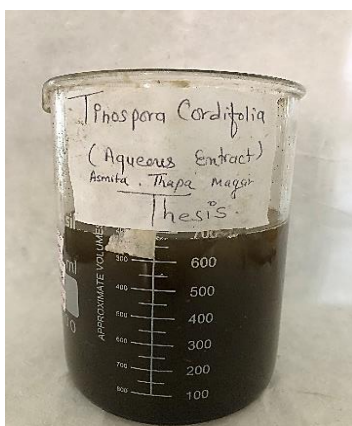
Rotary flask shaker



Well mixed solution



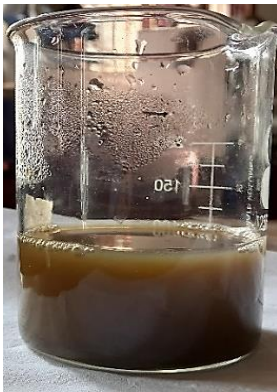
Filtration



Aqueous extract



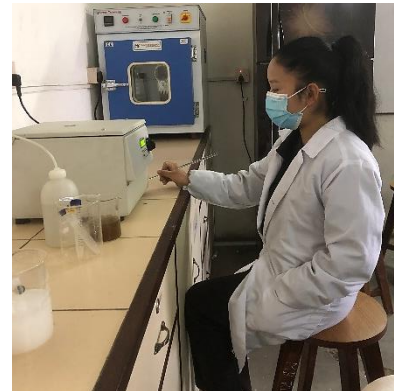
Reflux



**Refluxed solution**



**Centrifugation**



**Dialysis**



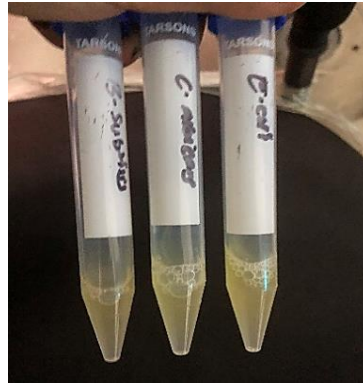
**Freeze drying**



**Freeze dried powder of  
Carbon dots**



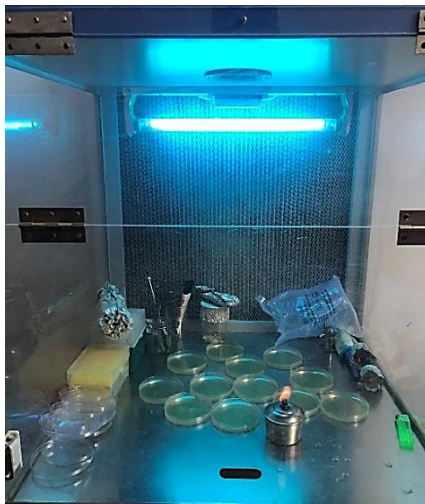
**Agar-agar solution**



**Bacterial solution**



**Sterilization**



**Agar-agar medium  
solidification**



**Incubation for 24 h**



**Antibacterial test**

# GREEN SYNTHESIS AND CHARACTERIZATION OF CARBON...

By: Asmita Thapa Magar

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## paper text:

GREEN SYNTHESIS AND CHARACTERIZATION OF CARBON DOTS USING TINOSPORA CORDIFOLIA STEM/LEAVES AND STUDY OF ITS ANTIBACTERIAL ACTIVITY A DISSERTATION

**SUBMITTED TO THE DEPARTMENT OF CHEMISTRY AMRIT CAMPUS INSTITUTE OF SCIENCE AND TECHNOLOGY TRIBHUVAN UNIVERSITY NEPAL FOR THE PARTIAL FULFILLMENT OF REQUIREMENT OF THE MASTER OF SCIENCE DEGREE IN CHEMISTRY**

BY ASMITA THAPA MAGAR SYMBOL No: 1871 T.U. REGISTRATION No: 5-2-33-243-2015 June, 2023

**BOARD OF EXAMINER AND CERTIFICATE OF APPROVAL This dissertation entitled**

, "Green Synthesis and Characterization of Carbon Dots using Tinospora cordifolia Stem/leaves and Study of its antibacterial activity" by Ms. Asmita Thapa Magar under the supervision of

Asst. Prof. Dr. Deval Prasad Bhattarai, Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu

, Nepal, and under co-supervision of Asst. Prof. Hari Bhakta Oli,

Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu, Nepal, hereby submitted has been approved for partial fulfillment of the requirement for completion of

her Master of Science (M.Sc.) Degree in Chemistry. This dissertation has not been submitted to any other university or institution previously for the award of a degree. .... Supervisor Asst. Prof. Dr. Deval Prasad Bhattarai Department of Chemistry Amrit Campus, TU, Kathmandu, Nepal  
..... Co-supervisor Asst. Prof. Hari Bhakta Oli Department of Chemistry Amrit Campus, TU, Kathmandu, Nepal .....  
Internal Examiner External Examiner ..... M.Sc. Chemistry Coordinator Assoc. Prof. Dr. Bhusan Shakya

Department of Chemistry Amrit Campus, TU, Kathmandu, Nepal ..... Head of the Department Assoc. Prof. Kanchan Sharma Department of Chemistry Amrit Campus, TU, Kathmandu, Nepal June, 2023 II RECOMMENDATION

This is to recommend that Asmita Thapa Magar, (Symbol. No. 1871., T.U. Registration No. 5-2-33-243-2015), has carried out a dissertation entitled "Green Synthesis and Characterization of Carbon Dots using *Tinospora cordifolia* Stem/leaves and Study of its antibacterial activity" for the requirement to the dissertation in Master of Science (M.Sc.) degree in Chemistry under my supervision in the Department of

Chemistry, Amrit Campus, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal

She has fulfilled all the requirements laid down by the Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal for the submission of the thesis for the partial fulfillment of the Master of Science (M.Sc.) degree in Chemistry. ----- Asst. Prof. Dr

. Deval Prasad Bhattarai Supervisor Department of Chemistry Amrit Campus Tribhuvan University Kathmandu, Nepal June, 2023 III DECLARATION This dissertation entitled "Green Synthesis and Characterization of Carbon Dots using *Tinospora cordifolia* Stem/leaves and Study of its antibacterial activity"

is being submitted to the Department of Chemistry, Amrit Campus, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal for the partial fulfillment of the requirement to the dissertation in Master of Science (M.Sc.) degree in Chemistry. This dissertation is carried out by me under the supervision of Assistant Prof. Dr