Biomass mediated synthesis of carbon dots from *Trigonella foenum graecumL* (stem) and *Borassus flabellifer* (fruit pod)

Ruthra.N

(16PCH015)

Thesis Submitted to

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore-641 043

In Partial Fulfilment of the Requirements for the Degree of

Master of Science in Chemistry

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10/4/18
Signature of the
Head of the department

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RUTHRA.N

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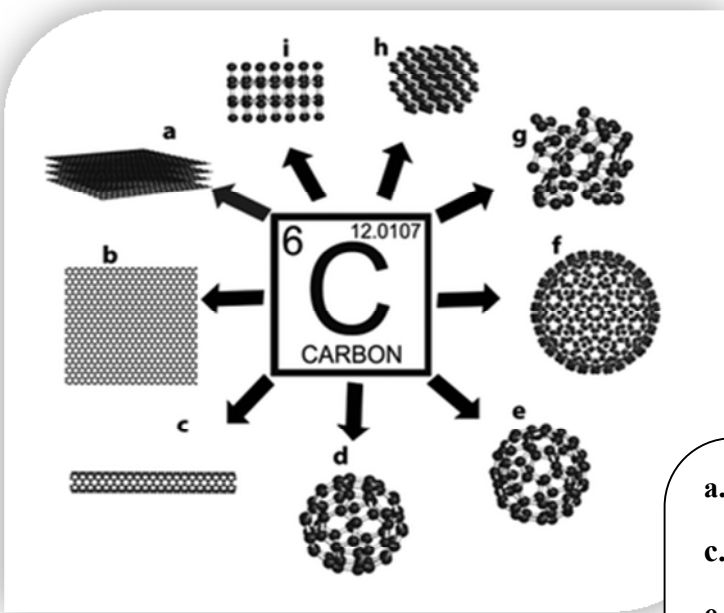
LIST OF ABBREVIATION

CNTs	Carbon nanotubes
CDs	Carbon dots
CVD	Chemical Vapor Deposition
SWNTs	Single-walled nantubes
MWNTs	Multi-walled nanotubes
QDs	Quantum dots
Cd	Cadmium
PL	Photoluminescence
CNPs	Carbon nanoparticles
CQDs	Carbon quantum dots
LED	Light-emitting diode
N-CDs	Nitrogen doped carbon dots
TF-CDs	Carbon dots from *Trigonella foenum graecumL (*stem)
BF-CDs	Carbon dots from *Borassus flabellifer*

INTRODUCTION

Carbon is an incredible material, known as “the king of the elements”. It is the element required for all life process and also it exists in many allotropic forms.



a. Graphite **b.** Graphene

c. Carbon nanotube **d.** C_{60}

e. C_{70} **f.** C_{540}

g. Amorphous solid

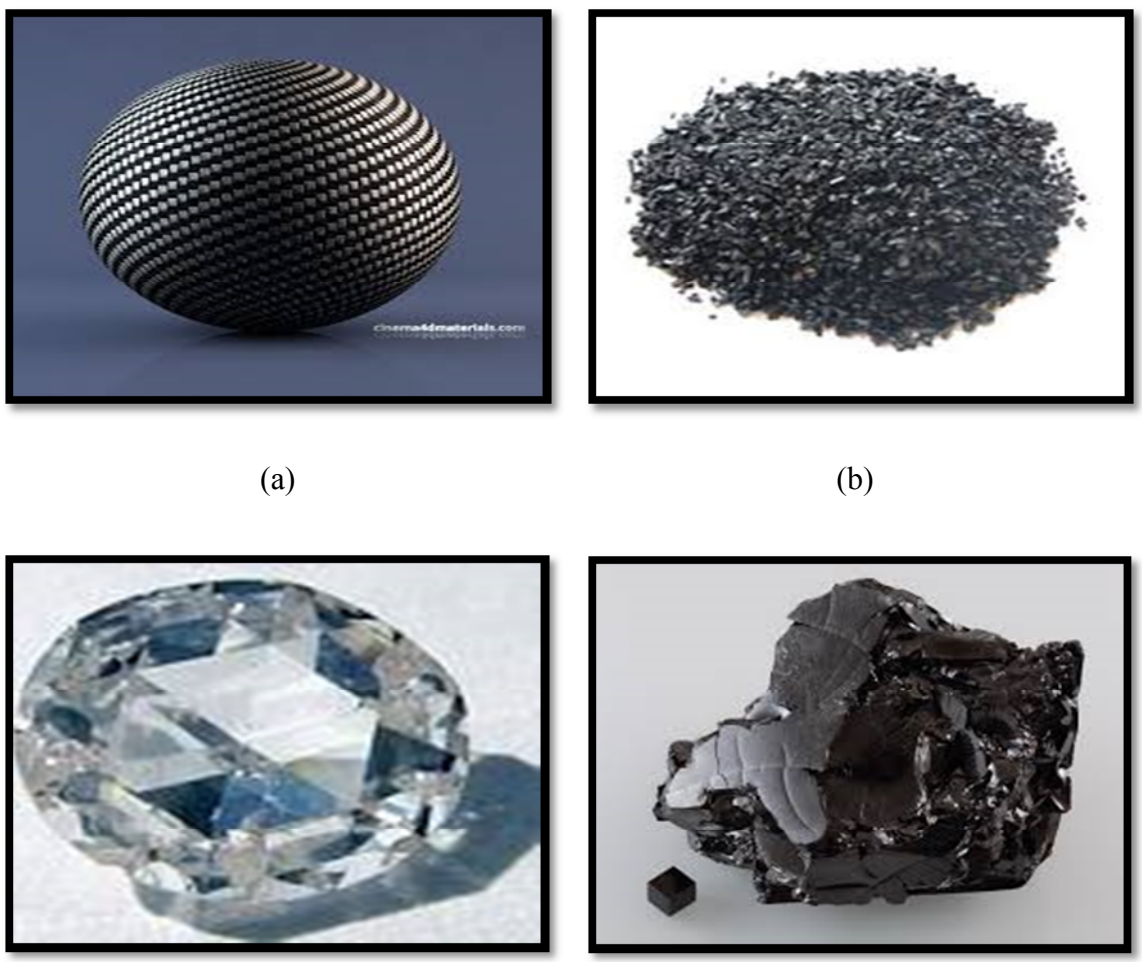
h. Lonsdaleite **i.**Diamond

Fig.1.1 Allotropes of carbon material (Ravula *et al*., (2015))

Carbon materials have come upon a hasty development since 1980s. Carbon materials of diverse class have been synthesized such as CNTs, fullerenes, graphene, CNFs, CDs and so forth. Recently different carbon materials of distinct nanostructures with tunable surface area, and pore size have been developed and they are prepared in a controlled manner. Their physical and chemical properties depend on crystalline structure. Diamond is a hardest, colorless, transparent and crystalline in nature. The electrical conductivity is very low because the energy gap between the valance band and conductance band is very large, but has good thermal conductivity whereas graphite is opaque, black in color and is soft. Graphite has good electrical conductivity but poor thermal conductivity but both the thermal and electrical conductivities increase with rise in temperature.

These carbon materials have various synthesis strategies like precursor-controlled pyrolysis, CVD, sol-gel method, nanocasting, and grafting methods which facilitates the high quality carbon materials.

Carbon materials, due to their physical and chemical properties, have been utilized in wide range of areas such as energy harvesting, storage and conversion, catalysis, nanocomposite materials, adsorption and separation. Bearing in mind the various environmental concerns, highly qualified, simple, innovative carbon nanostructures are being developed by scientists across the globe.



(a) (b)

(c) (d)

Fig.1.2 (a) Fullerenes (b) Activated carbon (c) Diamond (d) Glassy carbon (www.google.co.in)

1.1. Classification of carbon materials

Carbon materials are of various kinds they can be easily classified as technology grows. The different forms of carbon materials are tabulated below

Crystal /Micro Structure	Classic	Synthetic Conventional	Synthetic Nanotexture	Synthetic Nanosize	Orientation/ Symmetry
3D isotropic	Natural diamond	Diamond SiC			Bulk
3D anisotropic	Natural graphite	Artificial Graphite Pyrolitic Carbons	Flaky Graphites Pyrolitic Carbons Cokes		Planar
2D		C-C Composites	Intercalation Compounds Amorphous C Thin Films	Graphene	Film Thin film
1D		Carbon Fibers	Carbon Nanofibers	Carbon Nanotubes	Axial
0D		Carbon Blacks		Fullerenes Nanodiamond	Point
Amorphous or Disordered	Carbonaceous Materials Hydrocarbons	Activated Carbons Polymers	Diamond-like Carbons Glass-like Carbons		Random

Table 1.1: Structural and morphological classification of carbon materials, their synthesize and production

The conventional carbon material includes diamonds, graphite blocks and activated carbons. Among these graphite and diamond occurs naturally. Activated carbons are light carbon materials obtained from coal, wood, nutshells, etc. The newly developed carbon materials such as nanostructured carbon includes carbon fibers, glass-like carbon material and diamond-like carbon materials while the nanosized carbon materials comprises of graphene, carbon nanotubes (CNTs) and fullerenes. Graphene refers to a single atom thick layer of carbon atoms which forms a regular hexagonal web. CNTs are 100

times stronger than steel at one-sixth the weight. It conducts heat and electricity similar to copper. CNTs are classified as single-walled nanotubes and multi-walled nanotubes.

CNTs were discovered in 1991 by Iijima. These functional nanomaterials have a variety of unique and attractive properties. CNTs are 100 times stronger than steel at one-sixth the weight. It conducts heat and electricity similar to copper. They have been used in the fields of engineering plastics, polymers, displays, anti corrosion paints, thin films and coatings, transparent and non-transparent conductive electrodes, hydrophobic coatings and anti-static packaging. Currrently active research is ongoing in fields such as batteries, fuel cells, solar cells, advanced devices, optics, water desalination etc. CNTs exist as single walled carbon nanotubes and as multi walled carbon nanotubes.

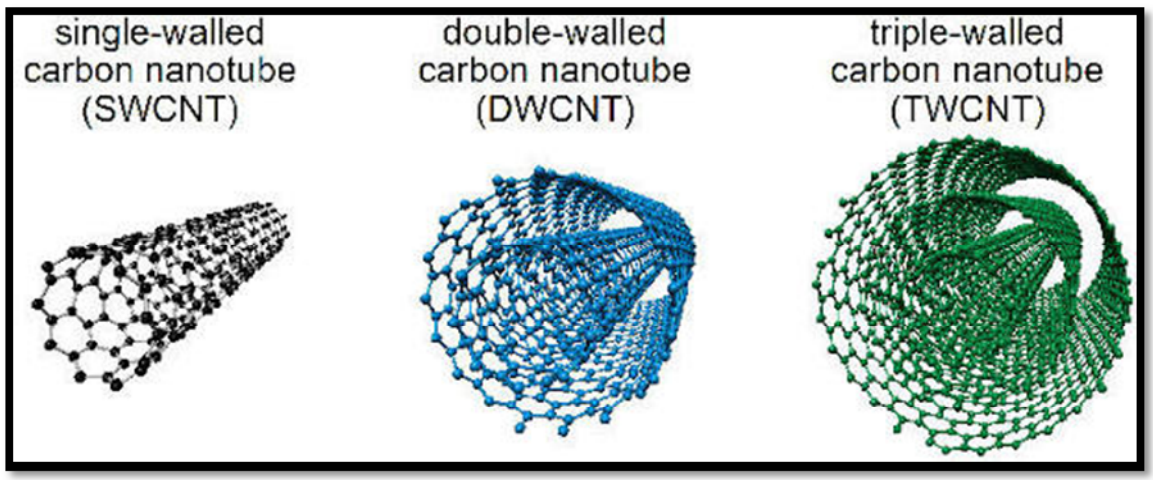


Fig.1.3.Different forms of nanotubes

1.3. Carbon Nanotubes Synthesis Methods

- **Arc discharge**

  The arc discharge method generally involves the use of a vacuum chamber and an inert gas supply. If the proper metal ions are introduced, even single-walled nanotubes can be produced. When optimized, this method can turn roughly 30% of the carbon into carbon nanotubes.

- **Laser ablation**

  Laser ablation has same mechanism as arc discharge, but laser is used as a substitute of heat through electrical discharge. A focused and powerful laser is used to promtly heat carbon and vaporizes it. The temperature and pulse times can be specifically controlled such that the parameters of the CNTs can be finely tuned. Nearly 70% of the carbon can be turned into CNTs.

- **Catalyzed Chemical Vapor Deposition**

Chemical Vapor Deposition (CVD) is the simplest method for producing CNTs. It involves the injection of vaporized hydrocarbon compound (methane or ethane are common) into a high temperature zone in a furnace. The hot zone contains a substrate on which has been pre-deposited a thin film of iron, nickel or cobalt that has either separated or been pre-patterned into nanoscale islands of the metal. This catalyzes the growth of the carbon nanotubes. Both single and multi-walled CNTs can be produced via CVD.

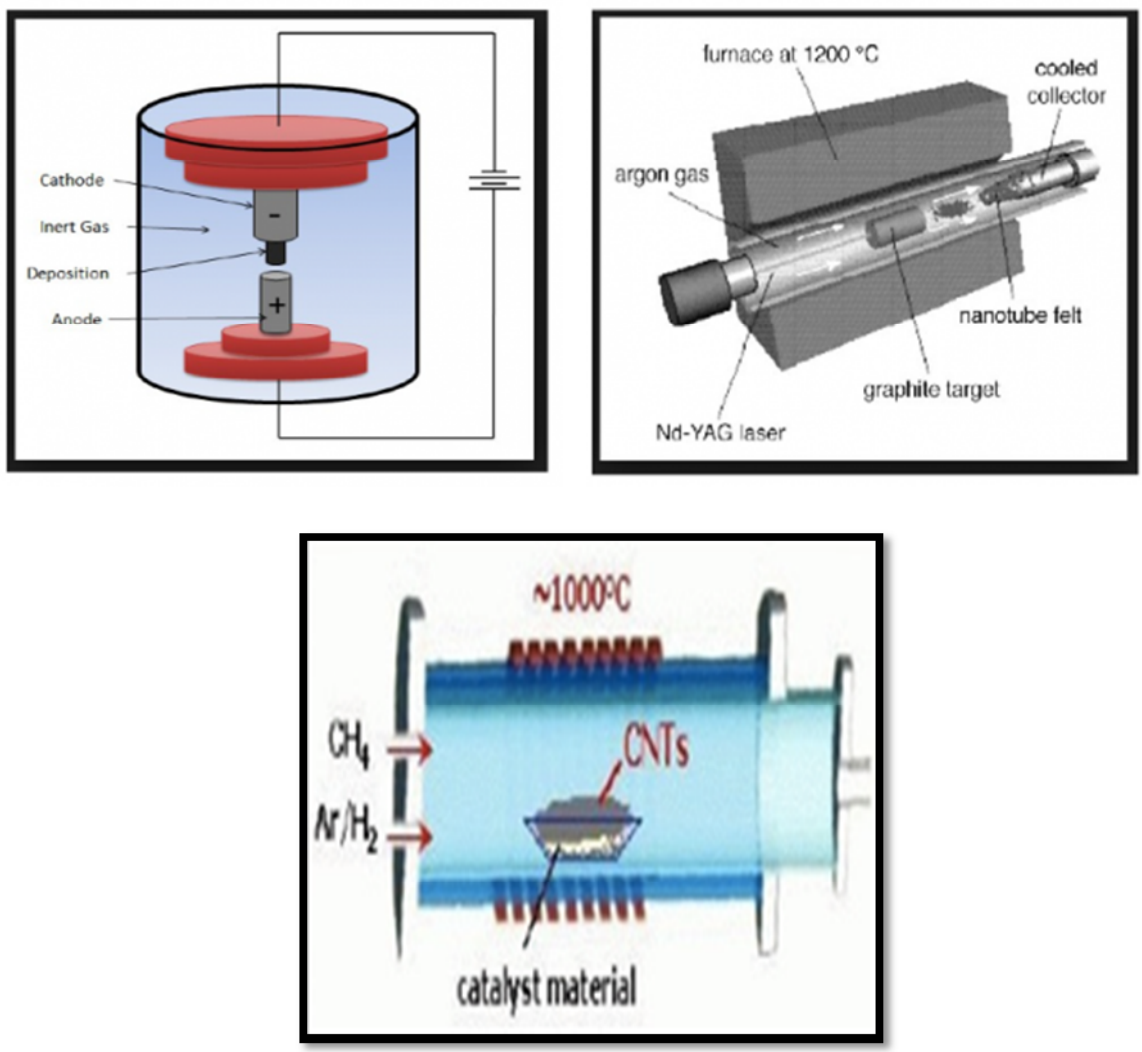


Fig.1.4. Arc discharge, Laser ablation and CVD for the synthesis of CNTs

1.3. Quantum dots and Carbon dots

Quantum dots (QDs) are generally semiconductor nanocrystals of physical dimensions smaller than the exciton Bohr radius. The electronic and optical properties of QDs depend on the nanoparticle sizes,

composition and on fluorescent colors.. The high optical performances of QDs have attracted the thoughts of researchers in biomedical fields, with the sighting of a varied potential bio-application. Despite the advantages of QDs for bio-imaging application they faced a major problem; among the most serious issue is the toxicity and they are limited to their applications owing to the use of heavy metals as essential elements which cause serious health issues and environmental hazards. For instance, Cd based QDs are very toxic to the vertebrate system even at comparatively low concentrations and also there may be a chance for the bioaccumulation of these toxic elements in the organs and tissues. For that reason researchers are keen in finding alternative QD-like photoluminescent materials. Among various nanoparticles, the development of nanosized silicon particles are found to be altrernative although they does not found to compete the cadmium based nanoparticles in the applications of bioimaging and other applications. But carbon which belongs the same group was found be the best alternative solution leading to the discovery of a particle with high fluorescence named carbon based quantum dots or carbon dots. (Baker *et al.*, 2010)

1.4. Carbon dots (CDs)

Carbon dots refer to a new class of carbon nanoparticle which sizes less than 10nm. They were discovered serendipitously during the purification of single walled carbon nanotube 2004 and later synthesized by sun *et al* in 2006. They are generally oxygenous carbon nanoparticles. In addition they found that this carbonaceous material could be fractionated into numeral components whose fluorescent properties depend upon their sizes.

This attracted the extensive concern of many scientific researchers, mostly because of its unique properties, low toxicity and stable chemical properties. As a result of this discovery they became a new probe for florescent thereby overcoming the earlier fluorescent probes.

CDs compared to quantum dots their size of carbon is only few nanometers and their molecular weight is also found to be comparatively low (few thousands to tens of thousands). CDs have large amount of –OH and –COOH on their surface which results in good water solubility and the suitability for subsequent functionalization with various organic, polymeric, inorganic, or biological species. CDs have unique optical properties as well, which have strong absorption in uv region. Their optical properties includes high fluorescent stability, nonblinking, tunable excitation, and emission wavelengths. The principle property of CDs is that they are good biocompatible and low toxic, since carbon material is the basic skeleton of all living body and thus CDs contains lower toxicity in comparision with other nanoparticles. Therefore it is very useful in biological fields. Their surface

contains lots of functional groups and can be adapted with further organic, inorganic, polymeric and other groups providing different functional properties.

1.5. Synthesis of CDs

The CDs can be prepared by either top-down method or bottom-up method. The synthesis of CDs is an easy process and also optimizes the luminescence properties. The top-down method is carried out by starting with a bulk scale materials and then scaling them down to the nanometer level dimensions. This involves physical breaking of the source material through high energy process. The bottom-up method is based on series of chemical reactions of small molecule precursor to form nanoscale particles.

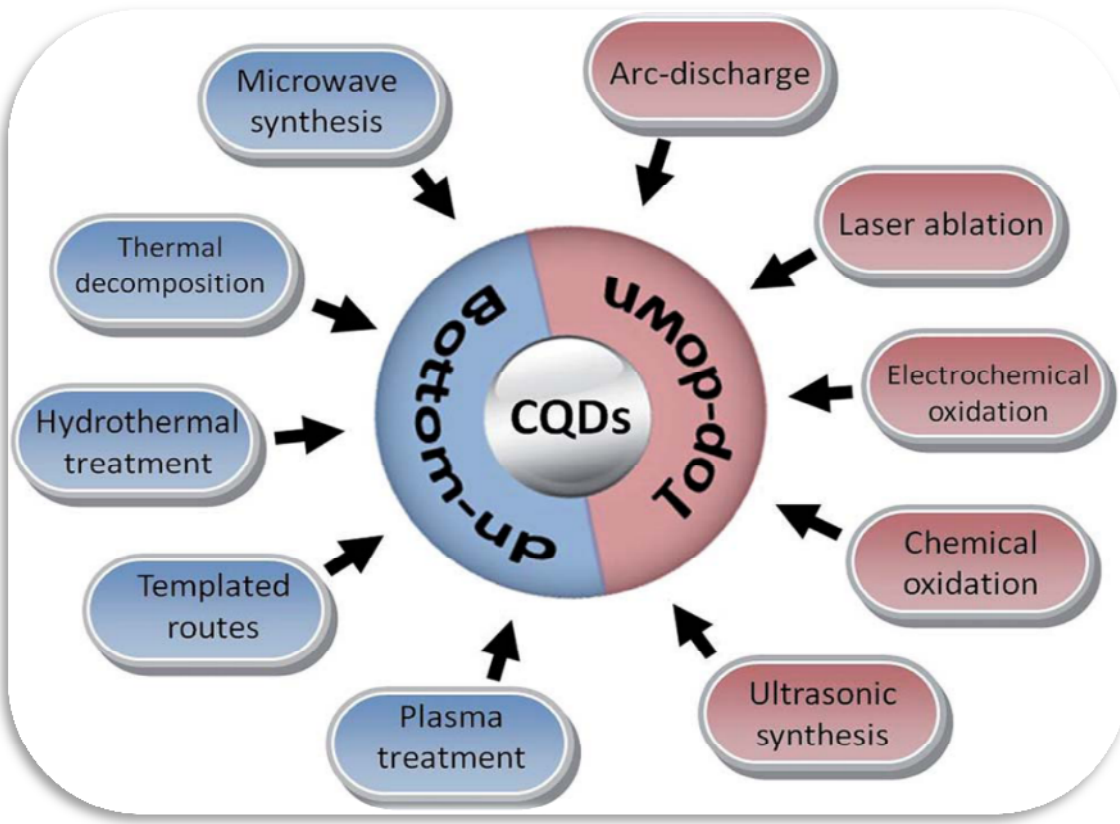


Fig.1.5. Schematic representation of synthesis of carbon dots through top-down and bottom-up approach (Wang *et al.*, 2017)

SYNTHESIS METHODS			
	Methods	**Description**	**Merits and Demerits**
Top-down method	Laser ablation	It is used for the removal of materials from a surface by irradiating intense laser pulse	Merits: Controllable morphology and size Demerits: Complicated operation, high cost
	Electrochemical oxidation	It involves transfer of electrons to or from a molecule or ion changing its oxidation state.	Merits: High purity, high yield, controllable size, good reproducibility Demerits: Complicated operation
	Chemical oxidation	It is the transfer of electrons from an oxidizing reagent to the chemical species being oxidized.	Merits: Easy operation, large scale production, no elaborate equipment Demerits: Non-uniform size distribution
	Ultrasonic treatment	The use of alternate low pressure and high pressure waves in liquids results in the formation of vacuum bubbles and this energy can be used to cut macro carbon particles to nanomaterial.	Merits : Easy operation Demerits: Instrumental wastage, high energy cost

Bottom-up method	Microwave synthesis	Microwaves impart intensive energy to detach the chemical bonds of a substrate. Due to its homogenous heating the size of the particle will be uniform.	Merits: Short reaction time, uniform size distribution, easy size control Demerits: High energy cost
	Thermal decomposition	The degradation and carbonization of organics takes place by external heating and forms nanoparticles.	Merits: Easy operation, solvent-free, low cost, large scale production Demerits: Non-uniform size distribution
	Hydrothermal treatment	At high temperature and pressure the precursors are potted inside a hydrothermal reactor.	Merits: High quantum efficiency, low cost, non-toxicity Demerits: Low yield

Table.1.2 Comparison of top-down a nd bottom-up methods

1.6. Hydrothermal method

The term “hydrothermal process” is defined as performing chemical reactions in solvents contained in sealed vessels in which the temperature of solvents can be brought to around their critical points via heating concurrently with autogenous pressures. This method is very useful for the synthesis of nanoparticles. It has many advantages over other conventional methods that include low processes temperature, performance of reactions in liquid environment, low energy consumption and environmental friendly.

Most of the hydrothermal methods are carried out in a sealed reactor. Autoclave, a pressure vessel is widely used. This pressure vessel is usually lined with Teflon or alloys or contains a tube made of gold, platinum, teflon or silver in order to protect the autoclave body from the corrosive nature of solvents at high temperature and pressure. Bourdon gauge is fixed to the autoclave which is useful to detect pressure directly.

In this present work hydrothermal method is chosen because other methods are more complex and require toxic chemical reagents whereas hydrotherrmal method is one-pot simple synthesis using economical carbon sources without the addition of any toxic chemicals and results in the formation of uniform particle size and highly stable carbon dots.

1.7. Applications of CDs

CDs have wide applications which include various fields like bio-imaging, bio-sensing, catalysis, optoelectronics, biological labeling, photocatalysis, temperature sensors, optoelectronic devices, biomedicine, delivering drugs, and fluorescent ink.

C-dot supported silver nanoparticles have been reported which showed improved efficiencies in polymer light emitting diodes and polymer solar cells

The combination of optical fiber technology with fluorescent C-dots results in improved sensitivity and fast response time.

C-dots have also been used in the fabrication of white LEDs which produced higher brightness.

Bio-sensing

The PL, electronic and electrochemical properties of CDs expose them for sensing applications with great potential. Since the size of CDs is similar to biomolecules they interact easily with biomolecules which leads to improved detection sensitivity.

Biosensors

CDs are biocompatible and serves as good electron acceptor or electron donor, thus it can be used for intracellular detection of ions, biological pH value, protein and enzymes, vitamins, and nucleic acid, etc. CDs were also used to detect ions such as Cu^{2+}, Hg^{2+}, Ag^{2+}, Cr^{3+}, Fe^{3+}, K^{+}, Cl^{-}, and H^{+}. Recently, CDs were used inside living pathogenic fungal cells to detect the intracellular pH value. CDs-based sensors have been developed to detect nucleic acid and have received wide interests to detect bio-molecules including vitamins and amino acids

Bioimaging

QDs limit their application in bio-fields due to the presence of heavy metals which are toxic. CDs act as a best alternative for this probe, as CDs have low toxicity and better photostability and are widely used in optical imaging. They are very good at bioapplications due to their visible excitation and emission wavelengths, high brightness at the individual dot level.

Drug/Gene Delivery

CDs acts as promising application in drug delivery systems and also used as anticancer agents.

Photocatalysts

The size-controlled C-dots show tunable emissions from near-infrared to blue wavelength, making them promising candidates as photocatalysts.

Energy and charge transfer

For fuel cell development or electrochemical energy storage, carbon with high surface area is instrumental in maximizing the performance of catalyst and energy systems. Increasing the surface area of carbon by fine particle dispersion or by using porous carbon has advanced the development of storage batteries and electrocatalysts for fuel cells.

1.8. Bio-synthesis of CDs

Till date there are plenty of reports which are successful in green synthesis of carbon dots. These bio-synthesized carbon dots replaced traditional quantum dots due to its low toxicity as it is using renewable natural sources as starting materials. CDs have also been synthesized from waste materials (wealth out of waste) thus reducing the pollutant level in the environment, making it eco-friendly. Since the sources used for the synthesis are natural sources, commercially available food and from waste materials, it is very cost effective too.

Many researchers have synthesized CDs from easily available sources such as coffee ground, milk, honey, orange juice, pomelo peel, Hylocereusundatus, banana, potato, garlic, lentinus edodes, cotton, prawn shells, watermelon peel, papaya, etc. the product obtained was high quantum yield and were found use in various biological applications.

In this regard the present work is undertaken to synthesize CDs from waste biomass ***Trigonella foenum graecumL*** **stem** and ***Borassus flabellifer*** **fruit pod.** Brief descriptions of the chosen species are as follows.

Plant description

Trigonella foenum-graecumL

Origin: Western Mediterranean native plant and spread throughout the Mediterranean region since ancient times.

Common name: Fenugreek

Species **:** *Trigonella foenum-graecumL.*

Kingdom **:** Plantae

Subkingdom : Viridiplantae

Class **:** Magnoliopsida

Family : *Fabaceae or Leguminosae.*

Subfamily : Papilionaceae

Genus : Trigonella L.

Order : Fabales

Habitat: roadsides, uncultivated land, dry grassland, wasteland and harvesting lands (grain fields)

Description of fenugreek: Fennugreek, *Trigonella foenum-graecumL* is an annual herbaceous plant of the leguminosae family, measuring between 20 and 50cm (upto 100cm) high. It is a member of bean family. It may have a single stem or branched-stem (at the base). The plant leaves are trifoliate with oval shape.

It produces pale white or purpilish flower and the seeds are brown in color. (http://plantvillage.org)

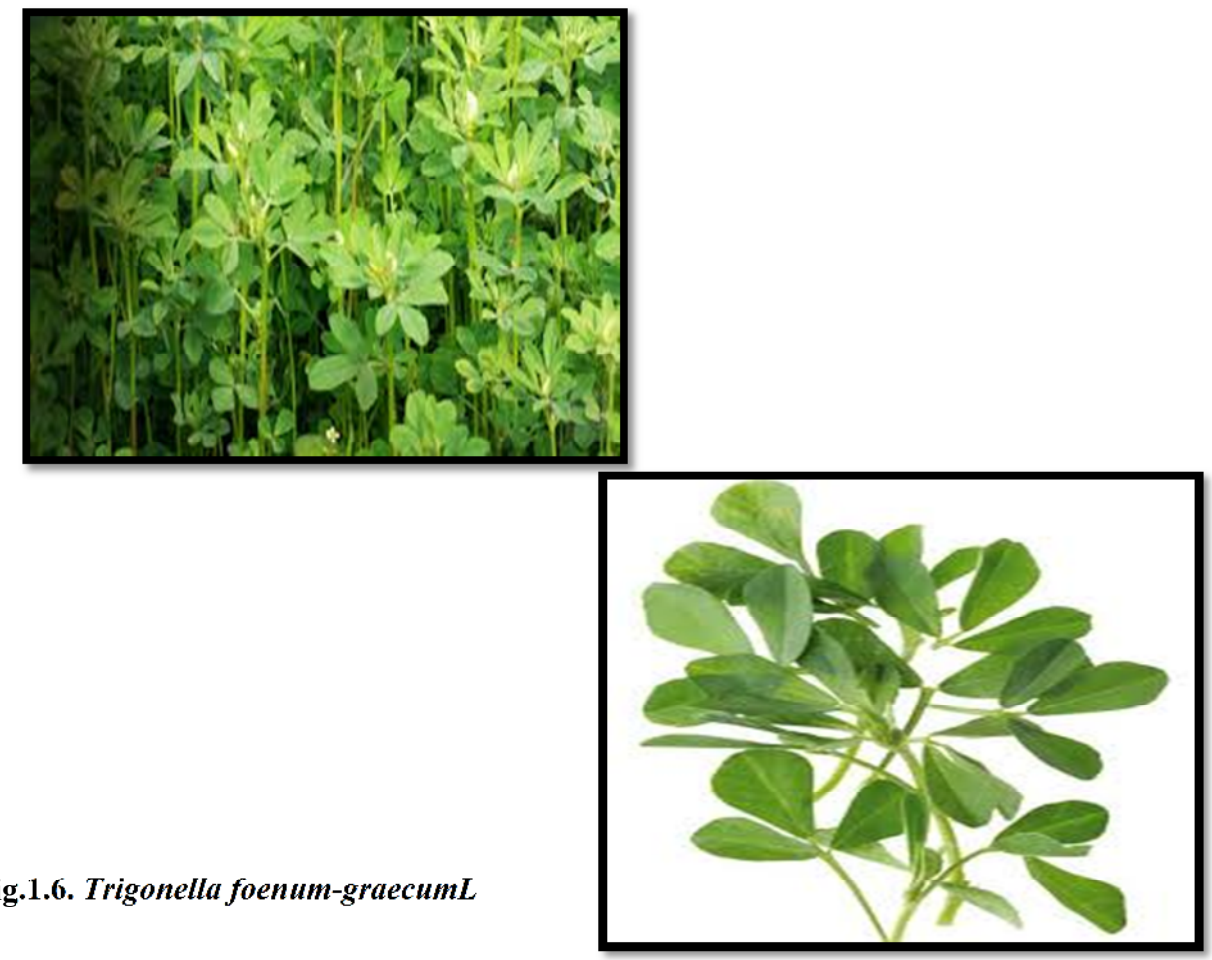


Fig.1.6. ***Trigonella foenum-graecumL***

Phytochemical constituents: Alkaloids, steroids, carbohydrates, terpenoides, flavonoids, quinones (Anbumalarmathi *et al*., (2016)).

Borassus flabellifer

Fig.1.7. ***Borassus flabellfer***

Scientific classification

Common name: Palmira palm

Kingdom **:** Plantae

Order **:** Arecales

Family **:** Arecaceae

Subfamily **:** Coryphoideae Griff.

Tribe **:** Borasseae Mart. ex Dumort.

Genus **:** *Borassus* L.

Plant description

It is commonly known as **doub palm**, **palmyra palm**, **tala palm**, **toddy palm** or **wine palm, found in** the Indian subcontinent and Southeast Asia, including India, Bangladesh, SriLanka, Cambodia, Laos, Burma, Thailand, Vietnam, Malaysia, Nepal, Indonesia and the Philippines.

Phytoconstituents

Gums, Saponins, Glycosides, Carbohydrates, Albuminoids, Fats, Vitamins A, B, and C. ((http://www.stuartxchange.org/Palmira.html)

1.9. Objectives

- To develop a simple and efficient method for the green synthesis of fluorescent carbon dots (CDs) from biomass waste namely ***Trigonella foenum-graecumL*** **(stem)** and ***Borassus flabellifer*** **fruit pod.**
- To characterize the synthesized carbon dots using UV-vis, FT-IR, Photoluminescence, 3D Optical profilometer, SEM.
- To study the anti microbial activity of the synthesized CDs

REVIEW OF LITERATURE

Carbon dots had drawn attention due to its unique optical properties, low toxicity and high biocompatibility. Many researchers have been working for the mass preparation of carbon dots and improved properties. CDs can be prepared by various methods but among them hydrothermal method was found to be utilized maximum because of its easiness. Since natural sources were used as precursors it was highly eco-friendly.

With this objective in mind, the facile synthesis of CDs from natural biomass carried out by several researchers is presented in review of literature to facilitate further understanding of the process.

They were found to have wide applications in biological imaging, sensing, drug delivery, optoelectronics, metal detection etc.

The applications of CDs are reviewed under the following headings

- ✓ Biomedical applications
- ✓ Sensor applications
- ✓ Optical applications
- ✓ Detection of metal
- ✓ Seed germination and growth
- ✓ Energy applications

Biomedical applications

- **Li *et al.,* (2010)** prepared CNPs by ultrasonic method using glucose as a natural precursor. CNPs were found to have stable and strong photoluminescence with NIR emission and up-conversion PL properties and have good application in bio-sensing and biotechnology.
- An amino-functionalized fluorescent carbon nanoparticles was formulated by **Yang *et al.,* (2011)** through simple hydrothermal treatment of chitosen as novel bioimaging agents without the usage of surface passivation agent and they were applied to bioimaging human lung adenocarcinoma A549 cells.
- **Mewada *et al.,* (2013)** produced carbon dots from *tropa-bispinosia* which have excellent fluroscent properties and quantum yield. They were found to be useful in delivery of active pharmaceutical ingredients and genes.

- **Saineelima *et al*., (2015)** used *Carica papaya* juice as a precursor for the preparation of carbon dots through hydrothermal method which emits multi -color emission. The synthesized carbon dots were also found to have biocompatibility and were used for imaging of bacteria and fungus cells.
- **Gu *et al*., (2016)** developed a microwave assisted synthesis of nitrogen doped carbon dots by lotus roots showing quantum yield of 19%. The synthesized carbon dots displayed application as a fluorescent probe for the detection of heavy metals and sensitivity on the detection of Hg^{2+} detection limit of 18.7 nM. Ultimately they are also used in cell imaging and sensing applications.
- **Miao *et al*., (2016)** build up a hydrothermal method for the synthesis of CDs from tomato juice. The as-prepared CDs showed a quantum yield of nearly 13.9%.It was used to evaluate the CEA levels in human serum, biological analysis and chemical determinations.
- Highly fluorescent CDs were synthesized using the leaves of tulsi via hydrothermal method by **Kumar *et al*., (2016)**. They exhibited good photostability and practically applied for lable-free sensing probe, detection of Pb^{2+} ions in triple negative breast cancer cells (MDA-MB 468 cells) and in water samples. They can also be applied in bioimaging.
- **Ensafi *et al.,* (2017)** demonstrated a facile synthesis of CDs through hydrothermal method by using saffron as starting material and were employed for the sensing of prilocanine and cell imaging.
- **Bhattacharya *et al*., (2017)** reported the synthesis of QDs/CDs with tunable excitation and emission properties using standard methods for the detection of tuberculosis volatile organic biomarkers
- A decomposition method was developed by **Wang *et al.,* (2017)** to attain carbon quantum dots from citric acid monohydrate as a carbon source and diethylene glycol bis (3-aminopropyl) ether as surface modification. The CDs were monodispersed with narrow size distribution and were used in cell/ tissue sensing.
- **Baig *et al*., (2017)** synthesized carbon dots from egg white through heating reaction and the size has found to be 3.3 ± 0.4nm, quantum yield (43%). They were found to be used as labeling agents for bacteria such as *staphylococcus aureus* and *Escherichia coli* and forster resonance energy transfer sensing for curcumin.

- **Yang *et al*., (2017)** prepared CDs from mushroom through hydrothermal method whose quantum yield was nearly 15.3% and were found that they showed good stability for detecting HA and Hase.
- **Dinc *et al*., (2017)** exhibited a microwave assisted synthesis of CDs from yogurt and found to have good dipersion with size of 2nm. They showed stable blue photoluminescence and are found to non-toxic on healthy cells CoN as well as on MCF-7 cancer cells upto 7.1mg/ml CD concentration. Therefore found applications in diagnosis and for intracellular delivery.

Sensor applications

- **Li *et al.,* (2010)** reported a one-step alkali assisted electrochemical fabrication of carbon quantum dots (CQDs). The obtained CQDs exhibits good photostability, up-conversion PL properties. They were found to be used as a powerful energy transfer component in photo-catalyst design, biosensor, bio-medical imaging and light-emitting diodes (LEDs).
- **Faisel *et al*., (2012)** reported hydrothermal method for the synthesis of CDs by using ascorbic acid and lysine, thus the water soluble carbon dots were found to highly biocompatible.
- Based on CQDs, a sensitive humidity sensing device was investigated by **Zhang *et al*., (2012)** through electrochemical ablation of graphite electrodes which confirmed high sensitivity to the environmental humidity and found promising application for sensing.
- **Tripathi *et al.,* (2014)** synthesized water soluble carbon dots from diesel soot through oxidative treatment by nitric acid. They were utilized for imaging $DH_{5\alpha}$ strain of E-coli and sensor for cholesterol detection and as well in drug delivery system.
- **Yuan *et al.,* (2015)** developed a hydrothermal method for the synthesis of CDs using wheat (biowaste) with 20% yield. The synthesized carbon were found to hold promising applications such as sensing ions, labeling and imaging inorganic nanostructure, cells and nematodes.
- **Phadke *et al.,* (2015)** prepared a biogenic synthesis of carbon dots using *Azadirachta* (neem) gum as precursor in ethanol and NaOH. Their size ranges from 5-10nm which were apt size for drug delivery system and biosensors.
- **Mehta *et al*., (2015)** reported the synthesis of CDs from apple juice via hydrothermal method with a quantum yield of 4.27%. They were found to have small oxygenous turbostratic and graphitic carbons domains with fluorescent properties and are highly biocompatible. They were used in the in vitro imaging of bacterial and fungal cells, drug delivery systems, bio-sensors etc.

- **Jhonsi *et al*., (2016)** fabricated a green synthesis of carbon dots from tamarind without additional stabilization whose quantum yield was found to be 4% with particle size of 1-3nm and have good storage stability. The electron transfer quenching of TCDs with acceptors was also expressed. The TCDs act well in bioimaging and sensing applications.
- Tumeric was used as natural precursor for the preparation of cobalt ferrite, carbon quantum dots and cobalt ferrite-carbon through hydrothermal method (**Alsmadian *et al*., (2017).** The quantum yield was found to be 20% and their effect of time and temperature on the morphology and particle size was investigated. The results confirmed that this method was suitable for the preparation and holds good for preparation of nnaocomposites and photocatalytic application.
- **Sun *et al*., (2017)** used *Lycii fructus* as a starting material for the synthesis of carbon dots by hydrothermal method. The synthesized carbon dots found applications in the detection of Fe^{3+} in urine and water sample with the limitation of 21nm and also as multicolor imaging and sensing applications. Their quantum yield was 17.2%.

Optical applications

- A microwave assisted synthesis of CDs using carbohydrate as a forerunner without use of surface passivation was reported by **Wang *et al.,* (2011).** They showed good biocompatibility, optical properties and imaging applications.
- **Dong *et al.,* (2012)** synthesized polyamine-functionalized carbon quantum dots (CQDs). They showed rapid detection, high sensitivity, and good selectivity, low cost and excellent application in detection of Cu^{2+} in water with detection limit as low as 6nM.
- **Zhou *et al*., (2013)** reported a solvent-thermal reaction for the synthesis of halogenated carbon quantum dots (CQDs) which provides an alternative approach for surface modification of CQDs.

Detection of metal

- **Zhu *et al*., (2013)** prepared a hydrothermal method for the synthesis of carbon dots. The reaction was carried out by condensation of citric acid and ethylenediamine which resulted in the formation of polymer like CDs, later carbonized to form CDs. The quantum yield was found to be 80%. The prepared CDs were used as printing inks, and functional nanocomposites that can be applied in anti-counterfeit, as well as utilized in the detection of Fe^{3+}.

- Bamboo leaves were used for the synthesis of carbon dots through hydrothermal method by **Liu *et al*., (2014)** and additionally capping was done using Branched polyethylenimine (BPEI) resulted in the formation of BPEI-CQDs. The quantum yield was 7.1%, with size of 3.6nm and utilized as a fluorescent probe in the detection of Cu^{2+}.
- Carbon dots were synthesized from *Jinhua bergamot* through hydrrotherrmal method by **Yu *et al.,* (2015)** and quantum yield was found to be 50.78%, fluorescence life time ca.384nm. They used for the detection of Hg^{2+} and Fe^{3+}.
- A facile synthesis of CDs with tunable frequencies using blue berry as a precursor via centrifuging and treatment with N_2 treatment was proposed by **Aslandas *et al*., (2015)**. They were sensitive in the detection of Fe^{3+} range from 12.5μM to 100μM. Lifetime measurements resolved the decrease in fluorescence lifetime of CDs in presence of Fe^{3+}.
- **Liu *et al*., (2016)** reported a hydrothermal method for the synthesis of N-CDs from rose-heart radish and the quantum yield was 13.6%. They were used for the detection of Fe^{3+} ions with a detection limit of 0.13 μM and cell imaging.
- **Vandarkuzhali *et al*., (2017)** prepared CDs via hydrothermal method using pseudo stem of banana. The quantum yield obtained was approximately 48% and they were found to have good recognition ability for Fe^{3+} ions. It was employed as a fluorescent probe for multicolor imaging.
- **Yang *et* al., (2017)** produced CDs from mangosteen pulp via calcinations in air which possess good water solubility and chemical stability. They were made use of for the detection of Fe^{3+} ions with detection limit of 52 nm and fluorescent probe for yeast cells.

Seed germination and growth

- **Park *et al*., (2014)** synthesized water soluble green carbon dots with high quantum yield from food wastes using ultrasonication method. The particle size ranges between (~ 4nm) which was confirmed by photoluminescence emission band (400-470nm) and the photoluminescence intensity was tend to decrease slowly under continuous excitation of Xe lamp for 10 days. It has low-toxicity, acts as a good probe for in-vitro bioimaging and the by-product formed was used in plant seed germination as well as growth.

Energy applications

- **Du *et al.,* (2014)** followed hydrothermal technique for the synthesis of CDs from orange pericarp. It showed narrow particle size distribution and good water solubility. The relationship among reaction time, reaction temperature and fluorescence capacity were also examined, the

results showed that, with decrease in particle size a blue shift occurred, fluorescent intensity increased with increase in reaction temperature or reaction time. Carbon microsphere was also obtained along with CDs. They were used in Li-ion batteries, catalysis, super-capacitors and hydrogen storage.

- **Zhang *et al*., (2015)** proposed a simpler one-pot green synthesis of CDs and C-coated metal (Au, Pt, Pd) particles using vitamin B_2 salts as carbon source and reducing agent. The prepared carbon dots showed both N and P doped characteristic.

From the literature review, many natural resources were found to be used as a precursor for the preparation of carbon dots with good quantum yields. Saffron, tulsi, blue berry, lotus root, Azadirachta (neem) gum , *Jinhua bergamot* (50.78%), wheat (biowaste) 20% yield, apple juice (42%), rose-heart radish (13.6%)., tamarind, *Lycii fructus,* banana stem(48%)

MATERIALS AND METHODS

In every research work the materials and methods employed are the features, which resolve the quantitative and qualitative facts of the product. In this present work, efforts have been taken to synthesize fluorescent carbon dots through simple one step hydrothermal method.

3.1. Selection of sample

Fenugreek (stem) and *Borassus flabellifer* (fruit pod) are naturally available and are widely spread. Both the fenugreek and *Borassus flabellifer* are commonly used in our day today life. Since they are naturally obtained they do not posses any environmental hazards in any kind. Keeping it in mind kitchen waste has been selected and was converted into a value added product. They are selected primarily on the basis of the following facts.

- Less expensive
- Possess no threat to the environment
- Non-toxic
- Easily available
- Bio-degradable
- Cost effective
- Eco-friendly

3.2. Preparation of sample

Fenugreek stem was collected from the kitchen, washed with clean water to remove dust/ muddy particles and are dried at room temperature for four days. The as dried stem was then grinded using grinder into powder and then used for synthesis.

The fruit pod of *Borassus flabellifer* was collected and cut into small pieces. It was then grinded into a fine thick paste without addition of water. It was made so because it may completely disperse on the addition of water such that maximum carbon dots can be extracted. They are then used for the preparation of carbon dots.

Phytochemical screening

Phytochemical examinations were carried out for both the extracts as per the standard methods **(Prashant Tiwari *et al*., 2011).**

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

4. Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

5. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

6. Detection of phytosterols

a) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of 2golden yellow colour indicates the presence of triterpenes.

b) Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

7. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

8. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

9. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

10. Detection of proteins and aminoacids

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

b) Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

3.3. Preparation of carbon dots

The powdered fenugreek stem was weighed 0.4g and added 10ml of distilled water. It was then kept in a Teflon-lined autoclave for 4hrs at 120°C until a yellow colored liquid was formed which indicates the formation of CDs. The solution was filtered and then centrifuged. **Wang *et al*., (2016)**

30ml of thick paste extracted from the fruit pod of *Borassus flabellifer* was taken and 40 ml of ethanol was added. The mixture was kept in Teflon-lined autoclave for 2 hours at 120□C until a dark colored solution was obtained (**Anbu *et al.*, 2017)**

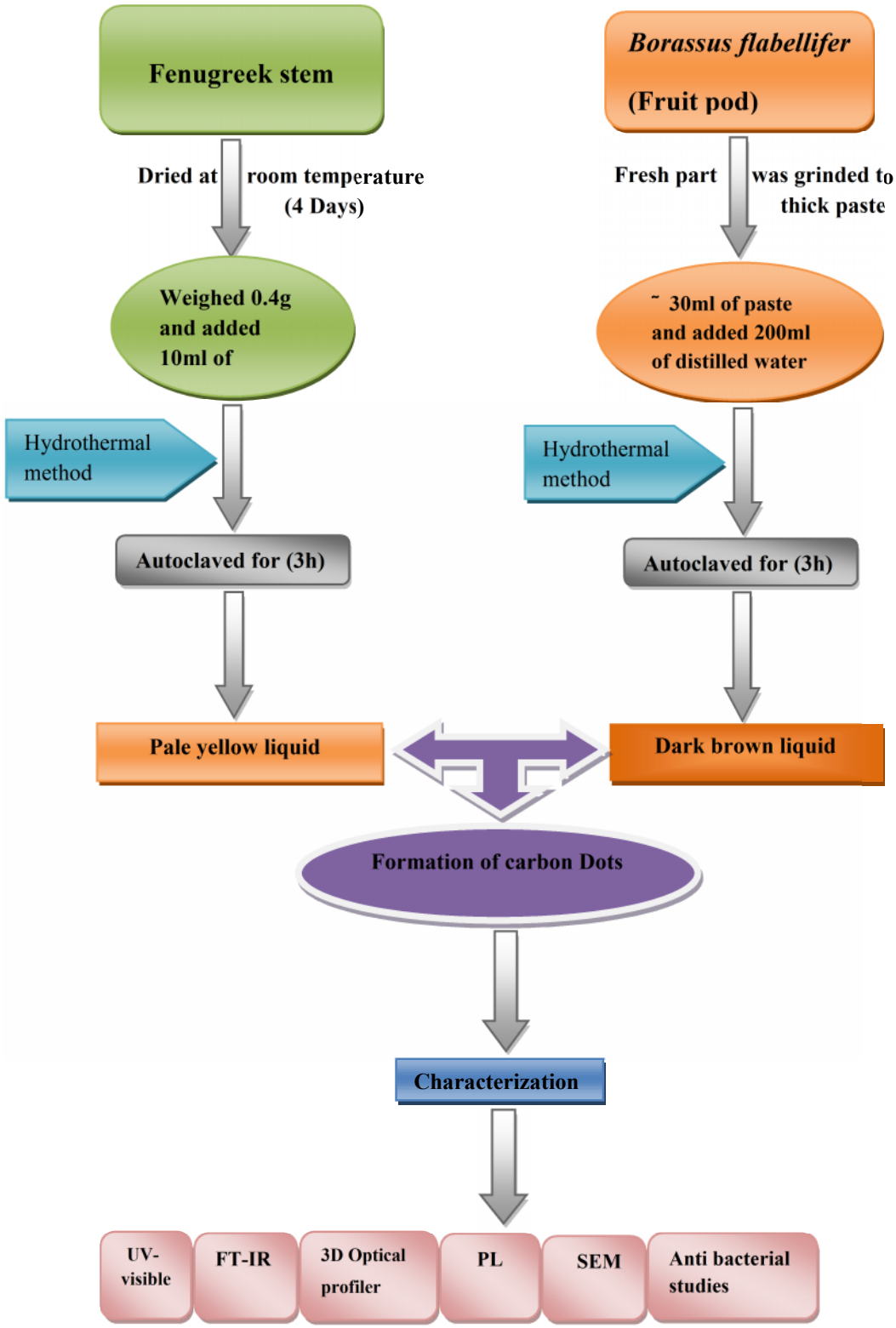


Fig. 3.1 Schematic representation of preparation of carbon dots

Characterization

UV-visible spectroscopy

PC based double beam spectrophotometer 2202 was used to confirm the possibility of the formation of carbon dots. UV-visible absorption spectrophotometric method was carried out on the synthesized carbon dots.

Band gap energy

The energy difference between the top of valence band to the bottom of the conduction band is referred as "band gap". The minimum amount of energy is required for the transition and that energy is known as the band gap energy.

The band gap energy of the sample was determined from its UV absorption spectrum.

Particle Diameter

Particle diameter of the synthesized carbon dots can be calculated with the use of a method described by Henglein and Coworkers using equation $2R_{CDs}= 0.1/(0.1338-(0.0002345 * \lambda_E))$.

The absorption wavelength is denoted as λ_e . By taking the absorption spectra of CDs as the wavelength at which the sharply decreasing region bends to be parallel to the X-axis, λ_E was determined. The particle diameter was denoted as 2R.

FT-IR Spectrophotometry

The samples for FT-IR studies were prepared by finely mixing the extract with spectroscopically pure KBr and then pressed by using a die so as to get a fine transparent pellet. The FT-IR spectrum was recorded for the identification of functional groups on the synthesized carbon dots in the range 4500 to 400 cm^{-1} using **Perkin Elmer FT-IR spectrophotometer** with the **SOFTWARE – OPUS version 6.5**.

PL Spectrophotometry

The photoluminescence spectra were recorded using Horiba JobinYvon fluoromax-4 spectrofluorometer. After continuous irradiation of UV light for different lengths of time, the fluorescent intensity of the synthesized carbon dots were measured on the fluorescence spectrometer under the same conditions.

3D Optical Profilometer

Surface profiles and pores were studied using a **Zeta-20 3D Optical Profiler**. The carbon dots (CDs) was mounted on sample holder occurred under the objective of the Optical Profiler and the 3D photos were taken from the 100x magnified surface via operating program on computer. The synthesized CDs were examined by Zeta 3 Dimensional Profiler.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) **JEOL MODEL JSM 6360** was used to examine the morphology of the prepared carbon dots. SEM was carried out to study the characteristics of carbon dots and to determine the dimensions of the synthesized carbon dots.

It uses a beam of electrons focused to a diameter spot of approximately 1nm in diameter on the surface of the specimen and scanned back and forth across the surface.

3.4. Antibacterial activity

The bacterial cultures were stocked at 4^0C after sub-culturing incubation of 24hrs at 37^0C in incubator. Mueller Hinton Agar were prepared and sterilized at 121^0C for 15minutes. The antibacterial assays were carried out by the agar well-diffusion method **(Jesteena *et al*., 2017)**. Mueller Hinton agar plate was prepared and after solidification 60 µl of culture was poured and spreaded with sterile cotton swab and kept for drying for 2-3 minutes. Wells were made with cork borer in the diameter of 5mm and added the TF-CDs and BF-CDs separately around 50µl in each well. Levofloxacin (LE 5mcg) antibiotic disc used as positive control. All the plates were incubated at 37^0c for 24 hours. The diameters of inhibition zone produced by the extract were measured in mm after the incubation period.

The cultures used for the study of inhibition efficiency of the prepared CDs were *Staphylococcus aureus*, *Escherichia coli,* and *B.cereus*

RESULTS AND DISCUSSION

The present work on **"Biomass mediated synthesis of carbon dots from *Trigonella foenum graecumL* (stem) and *Borassus flabellifer* (fruit pod)"** deals with the synthesis of carbon dots from *Trigonella foenum graecumL* (stem) and *Borassus flabellifer* (fruit pod) without use of any chemical reagents. The as-synthesized carbon dots were eco-friendly, cost effective and were considered a wealth out of waste initiation. The prepared carbon dots TF-CDs and BF-CDs were characterized by UV-visible spectrum, FT-IR, SEM, Photoluminescence, 3D Optical Profilometer and anti-microbial studies were carried out.

In order to understand the properties of CDs derived from sources that contain a variety of constituents in precursors, it is necessary to understand the type of constituents present in the precursors. Hence preliminary phytochemical studies have been carried out for the precursors and the results are discussed below.

4.1 Phytochemical screening

The precursors used for the preparation of TF-CDs and BF-CDs was screened for the presence of flavonoids, alkaloids, terpenoids, saponins, tannins, reducing sugar, phenols, glycosides and anthraquinones using standard procedures.(Mahmood *et al*., 2017)

Phytochemical screening of *Trigonella foenum graecum* stem

Nutrients	*Trigonella foenum graecumL*
Carbohydrate	++
Phenols	-
Catechol	-
Sterols	++
Alkaloids	++
Glycosides	+
Terpinoids	++
Saponins	+
Quinones	++
Anthocyanin	-
Tannins	+
Volatile oils	-

+ = Moderate presence ++ = Strong presence - = Absence

Table 4.1.1 Phytochemical constituents of TF-stem

Phytochemical screening of *Borassus flabellifer*

Test	Young fruit	Ripe seed coat	Cotyledon	Palm sugar
Reducing sugar	+	−	+	+
Terpenoids	+	−	+	−
Flavonoids	+	+	+	−
Saponins	−	−	−	−
Tannins	−	−	+	−
Alkaloids	−	−	−	−
Cardiac glycoside	−	−	−	−
Coumarins	+	+	+	−

Table.4.1.2 Phytochemical constituents of BF-fruit pod (Singchai *et al*., 2015)

The phytochemical screening indicated the presence of several phytoconstituents namely alkaloids, Flavanoids, Sterols etc. The presence of various functional groups like O-H, N-H, C=O, COOH, C-N etc., Similarly functional groups present in *Borassus flabellifer* fruit pod was also confirmed by FT-IR as seen in Fig.4.2.b and found to have C-OH, C-H, N groups etc. Both the carbonaceous extracts contain large amounts of hydrocarbons and oxygen groups which were suitable for the formulation of carbon dots.

UV-Visible spectrum of TF-stem

Fig.4.1 shows the UV-spectrum of the *Trigonella foenum graecumL* (stem) and the *Borassus flabellifer* (fruit pod). The characteristic absorption peak obtained for both the precursors were found to be 250 nm which may be attributed to π-π^* transition of aromatic sp^2 orbitals.

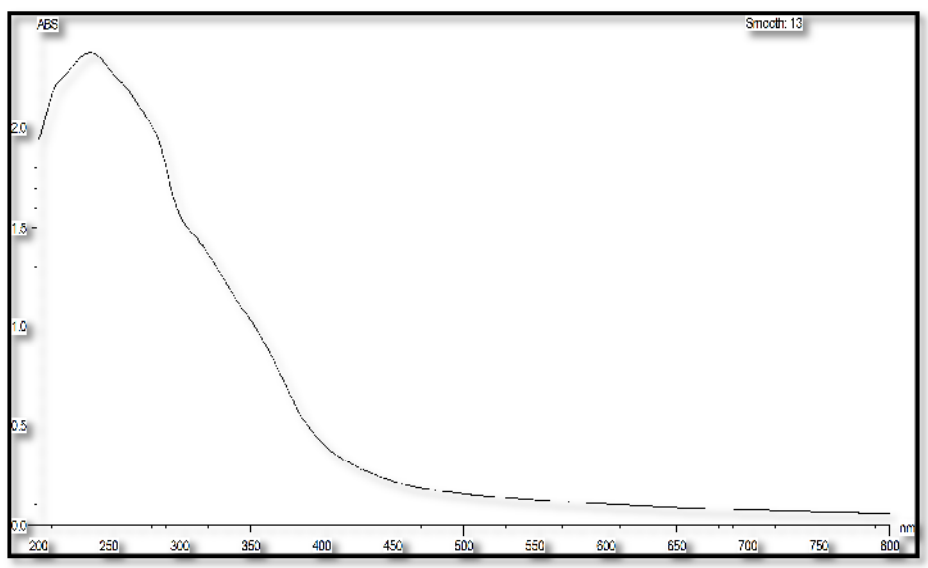


Fig.4.1.a UV-Visible spectrum for TF-Stem

Precursor	Absorption	Transition
Trigonella foenum graecumL (stem)	250 nm	$\pi - \pi^*$

Table.4.2.1 UV-Visible spectral peak for TF-Stem

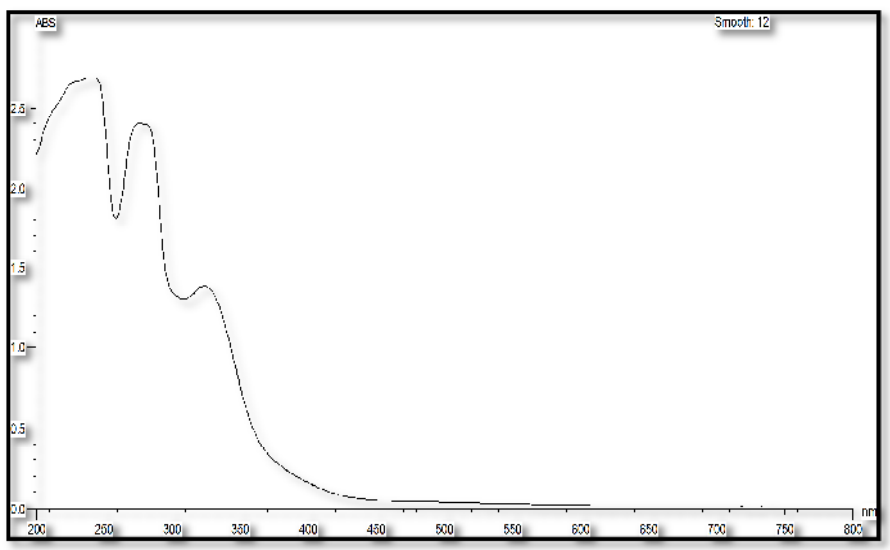


Fig.4.1.b. UV-Visible spectrum for BF-Fruit pod

Precursor	Absorption peak	Transition
Borassus flabellifer (Fruit pod)	250 nm	$\pi - \pi^*$

Table.4.2.2. UV-Visible spectral peak for *Borassus flabellifer* (Fruit pod)

FT-IR Spectrophotometry

***Trigonella foenum graecumL* (stem)**

The FT-IR spectrum of TF-Stem (Fig 4.2.a) indicated the presence of the following functional groups. An absorption band at 1099 cm^{-1} may be assigned with C-O bending vibrations. A medium absorption band appeared at 1242 cm^{-1} indicated the presence of C-O-C group. A peak at 1396 cm^{-1} might be attributed to the presence of symmetry and asymmetry vibrations of COOH groups whereas peaks at 1550 cm^{-1} , 1639 cm^{-1} and 1735 cm^{-1} was due to the presence of C-N, C=O & C=C bonds. C-H stretching, O-H & N-H stretching were observed at 2850 cm^{-1} to 2920 cm^{-1} & 3336 cm^{-1}.

***Brassus flabellifer* (fruit pod)**

As seen in Fig.4.2.b the absorption peaks for the BF-Fruit pod was observed at 1049 cm^{-1} and 1103 cm^{-1} indicated the presence of C-O bending vibration. Asymmetric and symmetric vibration of C-O-C was observed at 1249 cm^{-1} and a characteristic absorption of C-H bending vibration was found at 1442 cm^{-1}. Peaks at 1631 cm^{-1}, 2353 cm^{-1} and 3352 cm^{-1} was due to the presence of C=C stretching, & C=O, N related peaks and O-H stretching.

All these spectral data indicated the presence of functional groups like C=C, C=O, C-H, N and O-H groups in both *Trigonella foenum graecumL* (stem) and *Brassus flabellifer* (fruit pod).

Synthesis of carbon dots

Two types of green and natural precursors were selected for the synthesis of carbon dots, namely *Trigonella foenum graecumL* (stem) and *Borassus flabellifer* (fruit pod). The carbon dots synthesized after simple one step hydrothermal methods were found to be pale yellowish and dark brown in color which entailed the formation of CDs and subjected to the characterization studies.

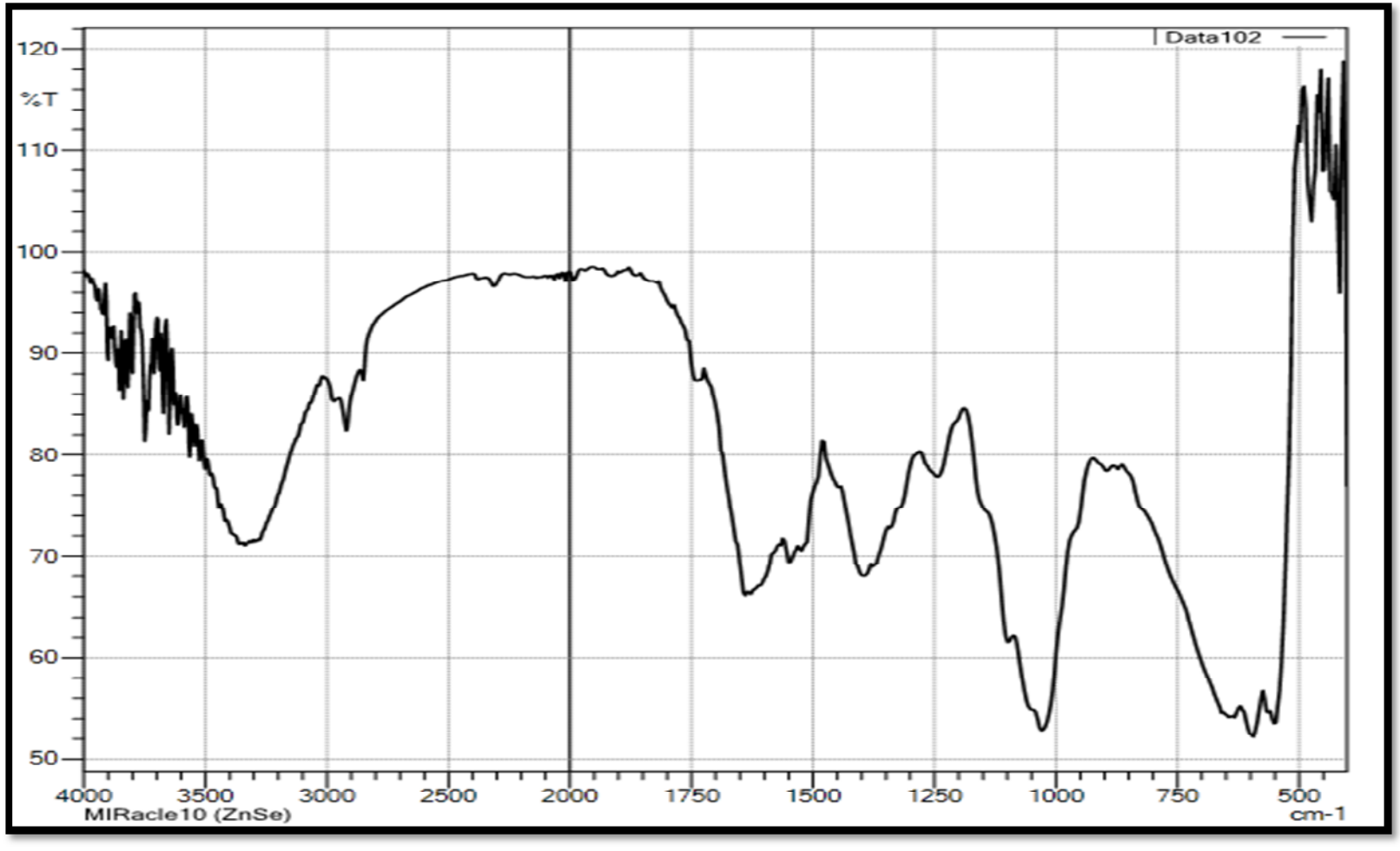


Fig.4-2.a. FT-IR spectrum of *Trigonella foenum graecumL* (stem)

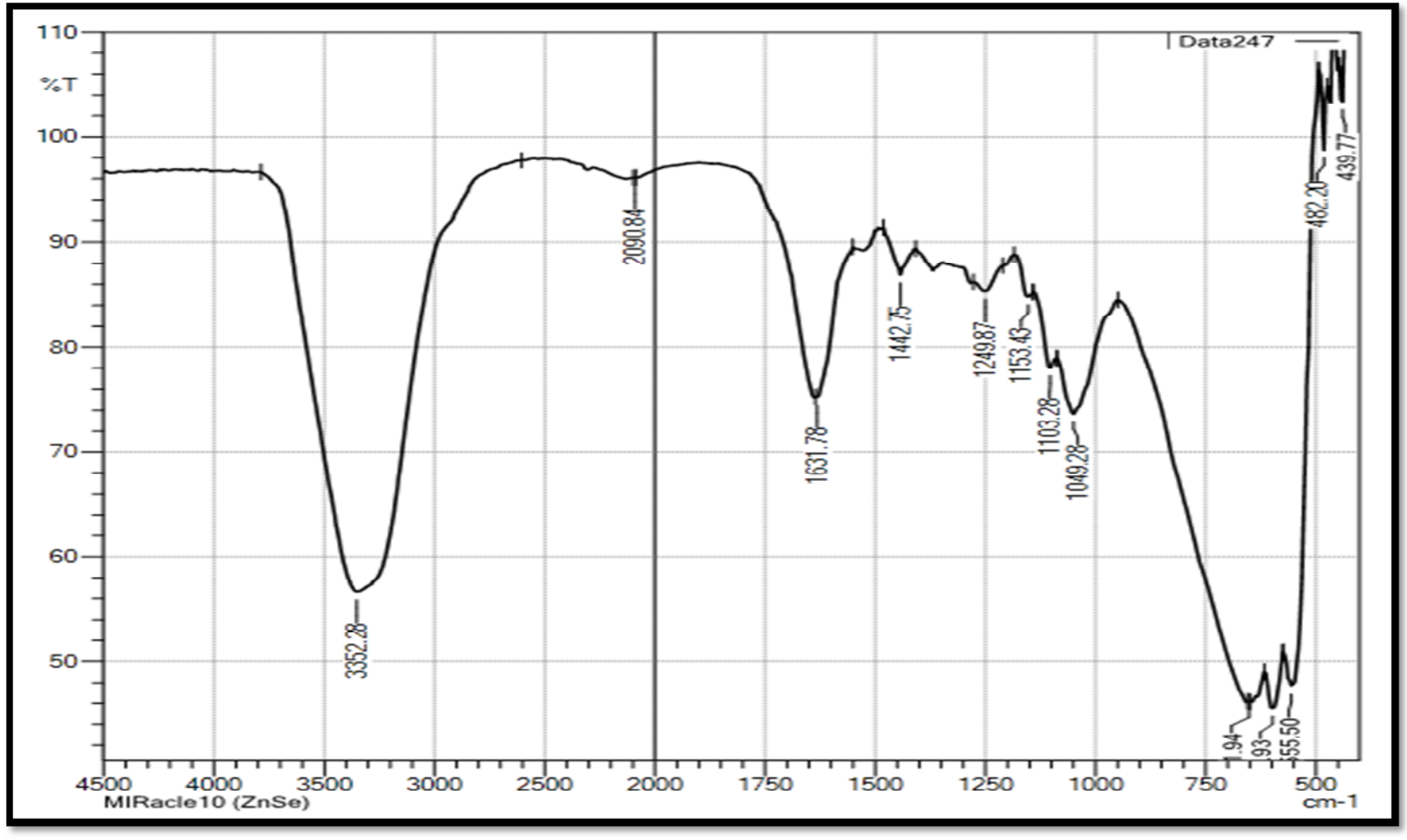


Fig. 4.2.b FT-IR spectrum of *Borassus flabellifer* (fruit pod)

4.2 Optical characterization

The optical characterization of both TF-CDs and BF-CDs were carried out through UV-visible spectrum and Photoluminescence to confer the formation of carbon dots.

4.2.1 UV-visible spectrometric studies

The UV-visible absorption was carried out for the synthesized carbon dots, TF-CDs & BF-CDs.

***Trigonella foenum graecumL* stem**

The UV-spectrum of the TF-CDs is depicted in Fig.4.3.a. The characteristic absorption peak for carbon dots obtained from *Trigonella foenum graecum* (stem) was observed at 223 nm, 267 nm and 307 nm which may be attributed to the π-π^* transition of aromatic sp^2 orbitals (Thambiraj *et al* 2016). The FG-CDs exhibited pale yellow color in day light, while exposed to UV light they showed blue fluorescence.

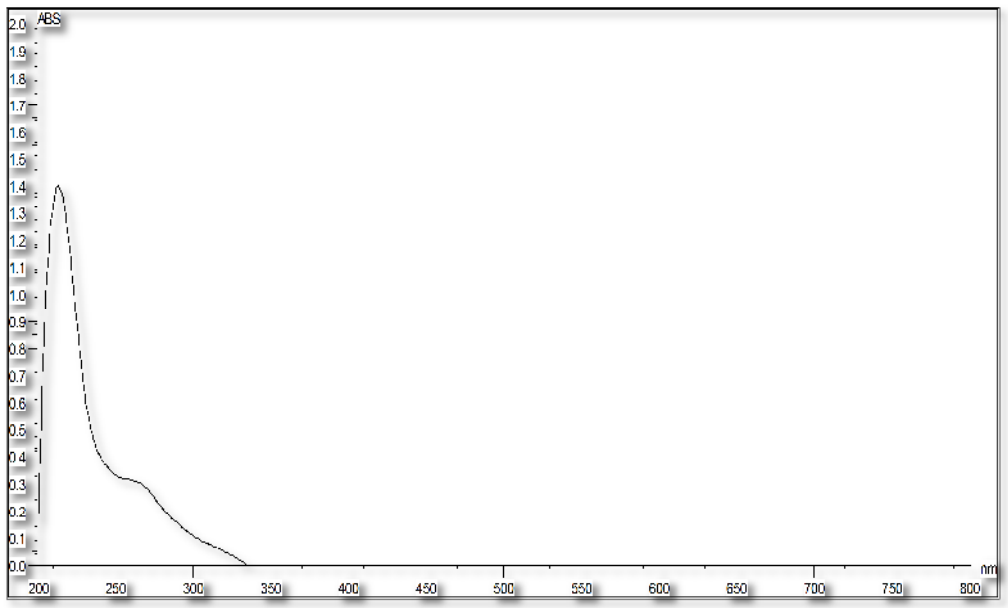


Fig 4.3.a UV- Visible spectrum of TF-CDs

Precursor	Absorption bands (nm)	
	TF-CDs	Transition
Trigonella foenum graecumL (stem)	223, 257 and 307	$\pi - \pi^*$

Table 4.3.1 UV-Visible spectral details for TF-CDs

***Borassus flabellifer* (fruit pod)**

The UV-spectrum of the synthesized carbon dots from *Borassus flabellifer* (fruit pod) is shown in Fig 4.3.b. The synthesized BF-CDs exhibited maximum absorption at 290 nm which may be attributed to π-π^* transition of aromatic sp^2 orbitals and another broad shoulder peak at 330 nm was from n- π^* transition of the C=O bonds originating from the surface of CDs (Jhonsi *et al*., 2016). They emit blue emission under UV lamp.

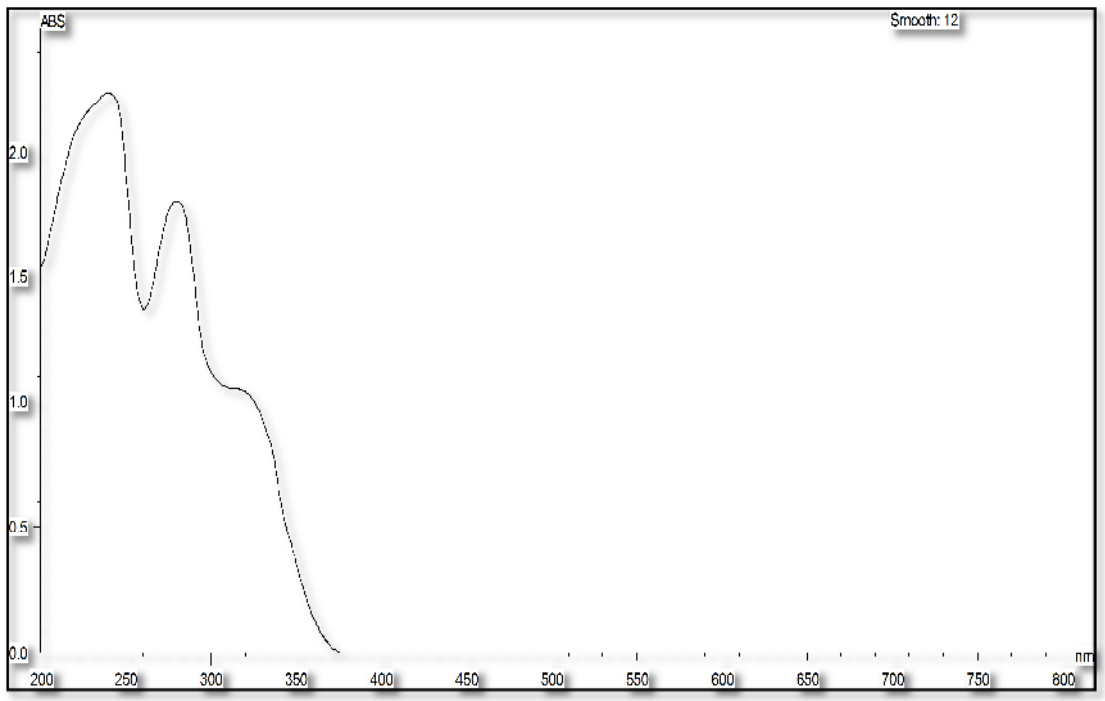


Fig 4.3.b UV-visible spectrum of BF-CDs

Precursor	Absorption bands (nm)	
	BF-CDs	Transition
Borassus flabellifer **(fruit pod)**	290 and 330	$\pi - \pi^*$

Table 4.3.2 UV-Visible spectral details for FG-CDs

Estimation of the CDs Particle Diameter

With the use of a method described by Henglein and Coworkers using equation

$$2R_{CDs} = 0.1/(0.1338-(0.0002345 * \lambda_e))$$

The CDs diameter was calculated with the use of wavelength at the edge of the UV absorption spectra (λ_e). The results are shown in table 4.4

Sample	Color	λ_e excitation (nm)	Calculated CDs diameter (nm)
TF-CDs	Bluish violet	223	1.22
BF-CDs	Blue	240	1.39

Table. 4.4.Particle Diameter of FG-CDs and BF-CDs

Determination of band gap

The spectral data recorded for TF-CDs and BF-CDs showed the strong cut off at 223 nm and 240 nm respectively, where the absorbance value is minimum.

The band gap was determined using the following equation,

$$E_g = h*C/\lambda$$

where $h = 6.626*10^{-34}$, is the planck's constant

$C=3.8*10^{8}$, is the velocity of light

λ is the cut off wavelength

Sample	Band gap in eV
TF-CDs	**5.56**
BF-CDs	**5.16**

Table.4.4.a Band energy gap for TF-CDs and BF-CDs

From the data it was clear that the as-synthesized CDs can be used in optical electronics due to their high band gap energy

4.2.3 Photoluminescence (PL) spectrometry

One of the fascinating properties of CDs is photoluminescence. On exposure to UV light the synthesized carbon dots exhibited blue luminescence in aqueous solution. The details from photoluminescence spectrometry were obtained from liquid CDs samples finely dispersed in water. The intensities were recorded for varied wavelengths from 270 nm to 620 nm with an interval of 20 nm. UV-visible range was used for the excitation of the samples.

PL of TF-CDs

Fig 4.4.a illustrated the emission for TF-CDs. The TF-CDs showed excitation PL emissions ranging from 290 to 600 nm which was completely excitation-dependent. Upon excitation at 290 nm, a maximum peak of 360 nm was noticed. When excitation was changed to 310 nm, the peak also shifted to 375 nm. The peak was found to be strong at 460 nm which was observed at excitation of 430nm. The variation in the emission peak may be attributed to the varied sizes of TF-CDs (Wang *et al*., 2014). Smaller particles get excited to longer wavelength and *vice versa*. The PL intensities also depend on the nature of surface of the CDs. Thus these two factors control the PL mechanism.

Difference in luminescence under UV light validates the formation of CDs. The fluorescence properties of the as-prepared carbon dots can be attributed to the presence of isolated sp^2 clusters and defect size within the 'C' matrix (Zhang *et al*., 2015)

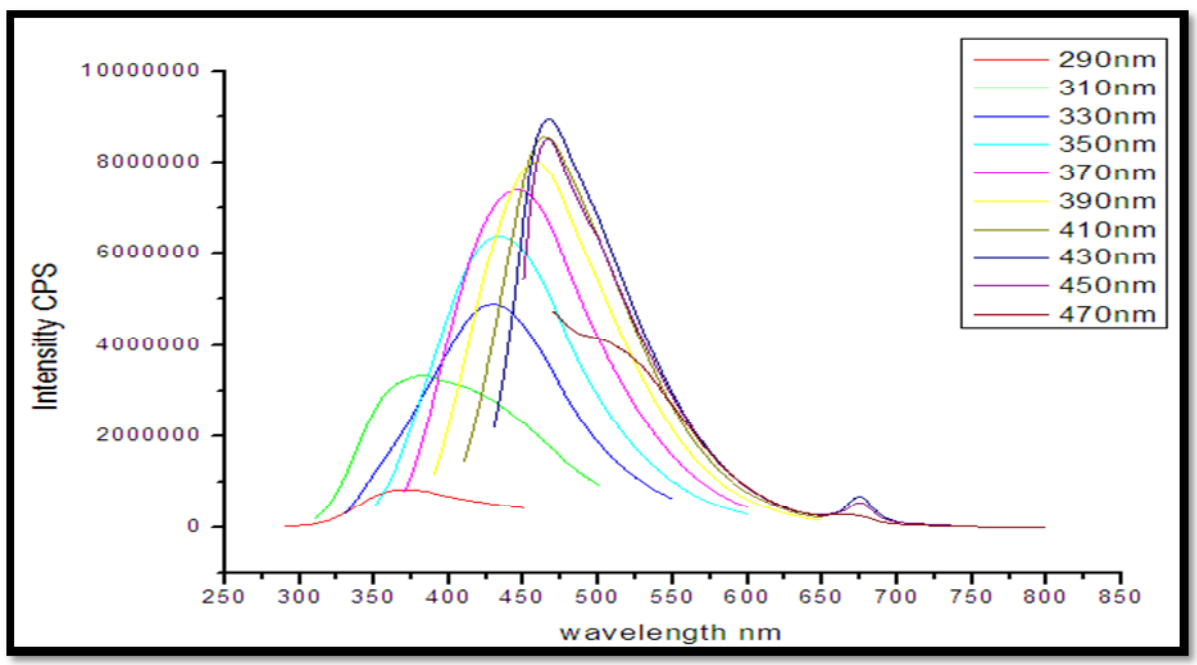


Fig. 4.4.a PL intensities of TF-CDs

PL of BF-CDs

The PL spectrum of BF-CDs (Fig 4.4.b) indicated a strong emission peak at 450 nm at excitation wavelength of 370 nm. This type of fluorescence band is observed generally for CDs (Aslandas *et al*., 2015). From the Fig.4.4.b. it can be noticed that the photoluminescence spectra are generally broad and dependant on the excitation wavelengths. The property of PL intensity depended on the particle size of the carbon dots i.e., size of BF-CDs and their surface nature. The functional groups present on the surface of BF-CDs may lead to the emission.

When the excitation wavelength varied from 300 to 460 nm in 20 nm increments, it is observed that emission maxima varies from blue to green region (380–520 nm), this bathochromic shift in emission wavelength with decrease in fluorescence intensity may be attributed to the different functional groups such as ketonic, carbonyl and hydroxyl groups present in the surface of carbon dots, which is consistent with the FT-IR measurement. Here, it is noted that the excitation dependent emission may be associated with the anti-stokes emission of BF-CDs and surface energy trap is thought to be the reason for the strong emission. This result is consistent with the previous reports for carbon dots from green synthesis (Jhonsi *et al*., 2016)

Therefore a controlled mechanism of PL can be obtained depending on the particle size and nature of the surface.

It was clear that the emission peaks of both TF-CDs and BF-CDs shifted to longer wavelength with increase in the excitation wavelength in conjugation with gradual decrease in PL intensities.
Such observations are similar to that of CDs which may be attributed to the optical selection of differently sized nanoparticle or different emission traps on the CDs surface (Wang *et al*., 2017)

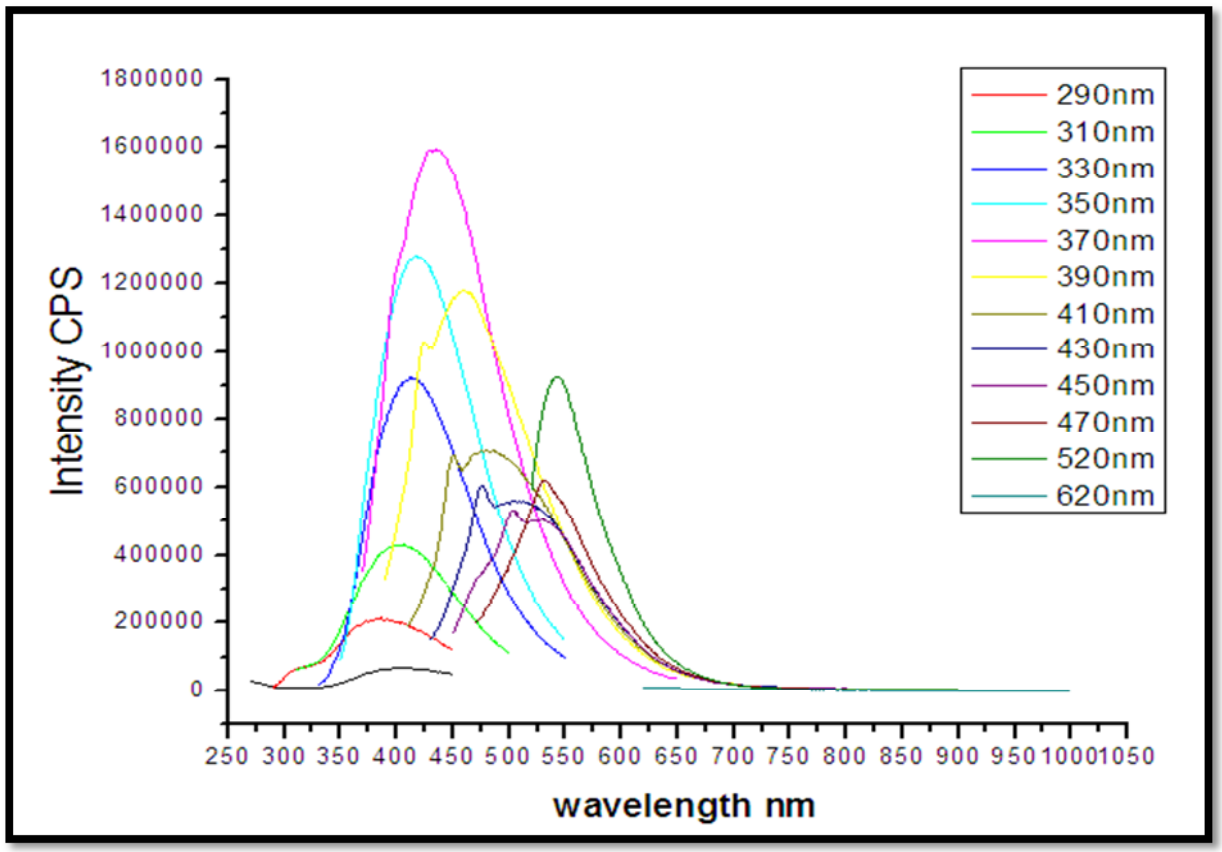


Fig 4.4.b PL intensities of BF-CDs

4.3 Surface analysis

The surface composition and morphologies of the synthesized carbon dots were analyzed by FT-IR, 3D Optical profilometer, and SEM.

4.3.1 FT-IR Spectrophotometry

FT-IR spectrum for *Trigonella foenum graecumL* (stem), *Borassus flabellifer* (fruit pod) and water soluble CDs were carried out. Several functional groups present on the surfaces of the synthesized carbon dots were studied.

***Trigonella foenum graecumL* stem (TF-CDs)**

The FT-IR spectrum of TF-CDs (Fig 4.5.a) indicated the presence of the following functional groups. An absorption band at 1045 cm^{-1} may be associated with C-O bending vibrations. A medium absorption band appeared at 1469 cm^{-1} which indicated the presence of C-C group. A peak at 1415 cm^{-1} might be attributed to the presence of C-N & C-C groups whereas peaks at 1585 cm^{-1} was due to the presence of C=O. N related peaks, O-H & N-H stretching were observed at 2353 cm^{-1}& 3217 cm^{-1}. In addition ,CDs display the characteristic bands for C=C and C-O-C vibrations at 1631 cm^{-1} and 1045 cm^{-1} respectively indicating the presence of sp^2 orbitals (Vandarkuzhali *et al*., 2017)

Powdered *Trigonella foenum graecumL* (stem)			TF-CDs		
Frequency cm^{-1}	**Assignment**	**Functional Groups**	**Frequency** cm^{-1}	**Assignment**	**Functional Groups**
1099	C-O bending vibration	Secondary alcohol	1045	C-O	Anhydride
1242	C-O-C	Alkane	1469	C-C	Alkane
1396	Symmetric and asymmetric vibration of COOH	Carboxylic	1415	C-N & C-C	Aromatic compounds
1550	C-N	Nitro compounds	1585	C=O	Ketone
1639,1735	C=O & C=C	Alkene	1631&1712	C=O & C=C	Alkene , conjugated aldehyde
2850-2920	C-H stretching	Alkane	2353	N related peaks	Nitro compounds
3336	O-H & N-H stretching	Alcohol & amine	3217	O-H & N-H	Alcohol

Table 4.5.1 FT-IR spectral peak of TF-CDs

Thus we can say that the as-prepared ‘CDs’ contain C=O, N-H and COOH group on the surface.

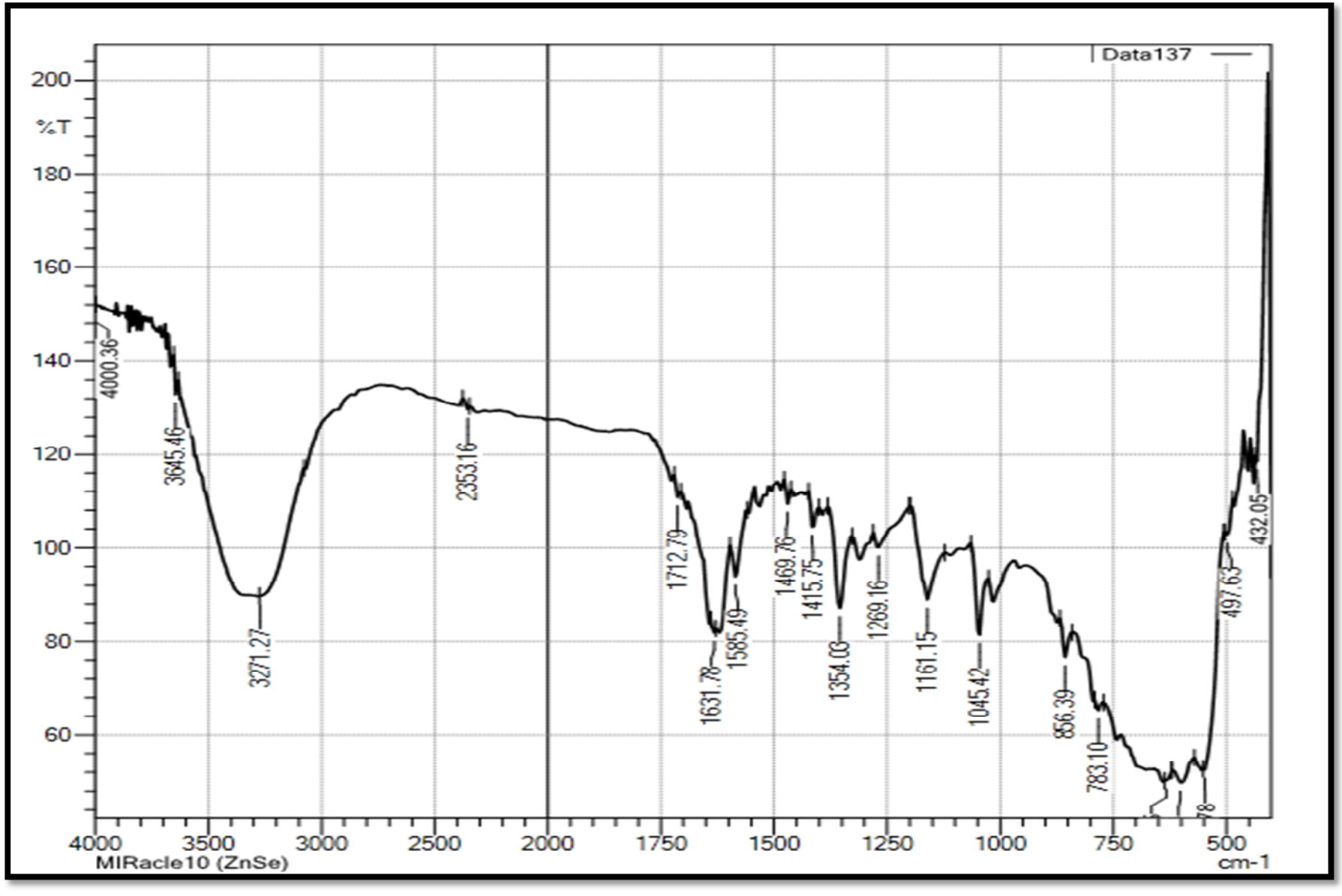


Fig.4.5.a FT-IR spectrum of TF-CDs

***Borassus flabellifer* (BF-CDs)**

As seen in Fig.4.5.b. the absorption peaks for the prepared BF-CDs was observed at 1053 cm^{-1} which indicates the presence of C-O stretching. Medium C-H bending was observed at 1450 cm^{-1} and a characteristic absorption at 1631 cm^{-1} was due to the presence of C=C stretching & C=O groups. O=C=O stretching, broad O-H stretching was identified at 2303 cm^{-1}, 3325 $cm^{-}1$ and 2684 cm^{-1}.

These peaks alternate the existence of hydroxyl and carbonyl group on the surface of CDs. Consequently the CDs are hydrophilic and stable in aqueous solution due to their functional groups containing oxygen

***Borassus flabellifer*(fruit pod)**			**BF-CDs**		
Frequency cm^{-1}	**Assignment**	**Functional Groups**	**Frequency cm^{-1}**	**Assignment**	**Functional Groups**
1049	C-O bending vibration	Alkyl aryl ether	1053	C-O stretching	Vinyl ether
1103	C-O bending	Alkane	1450	Medium C-H bending	Alkane
1249	Asymmetric & symmetric vibration of C-0-C	Carboxylic group	1631	C=C stretching & C=O	Cyclic alkene
1442	C-H bending vibrations	Alkane	2303	O=C=O stretching	Carbon di-oxide
1631	C=C stretching & C=O	Cyclic alkene	2684	Broad O-H stretching	Alcohol
2353	N related peaks	Nitro compounds	3325	O-H stretching	Alcohol
3352	O-H stretching, N-H	Alcohol, Secondary amine	3846	O-H	Alcohol

Table 4.5.2 FT-IR spectral peak of BF-CDs

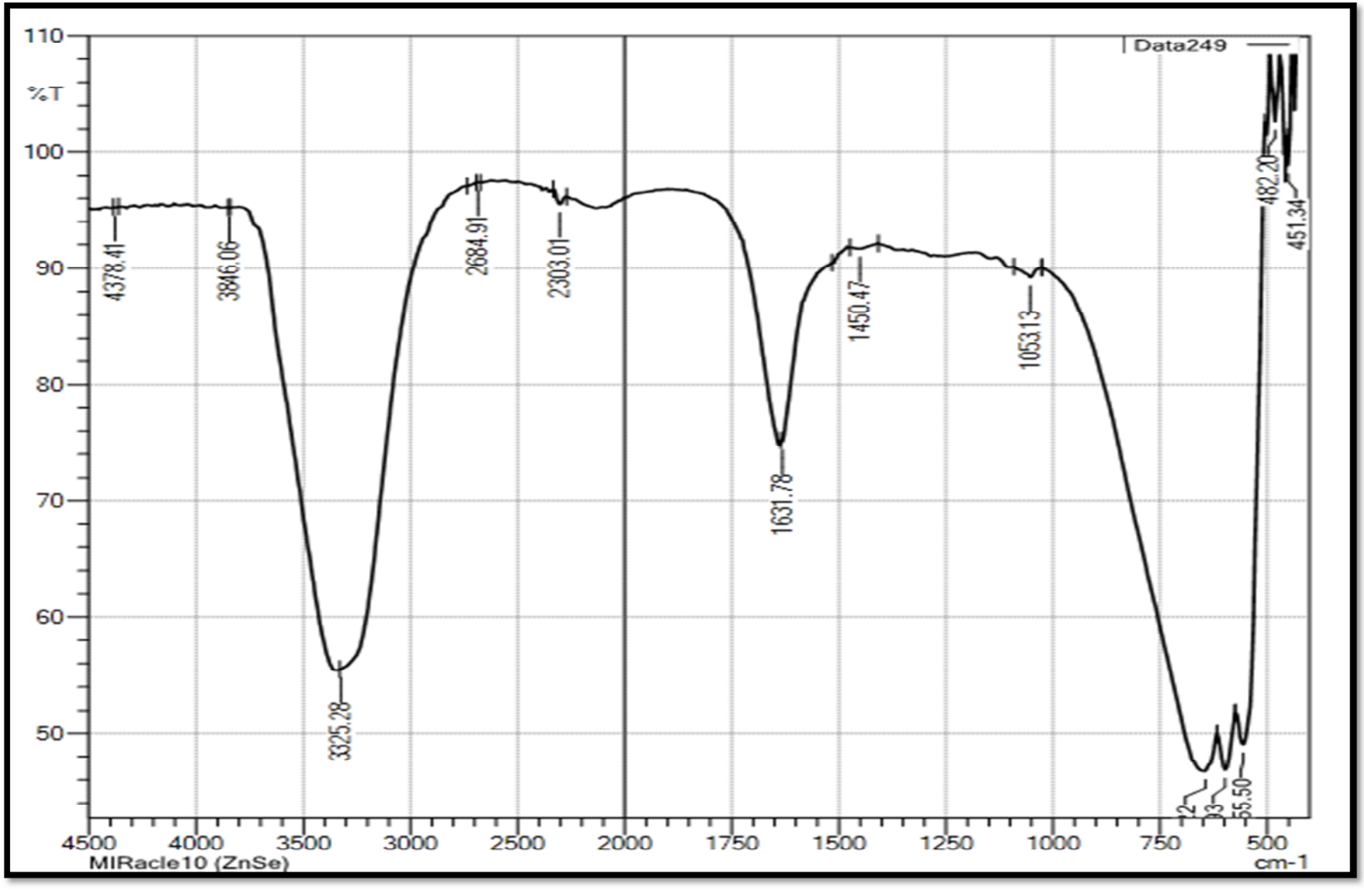
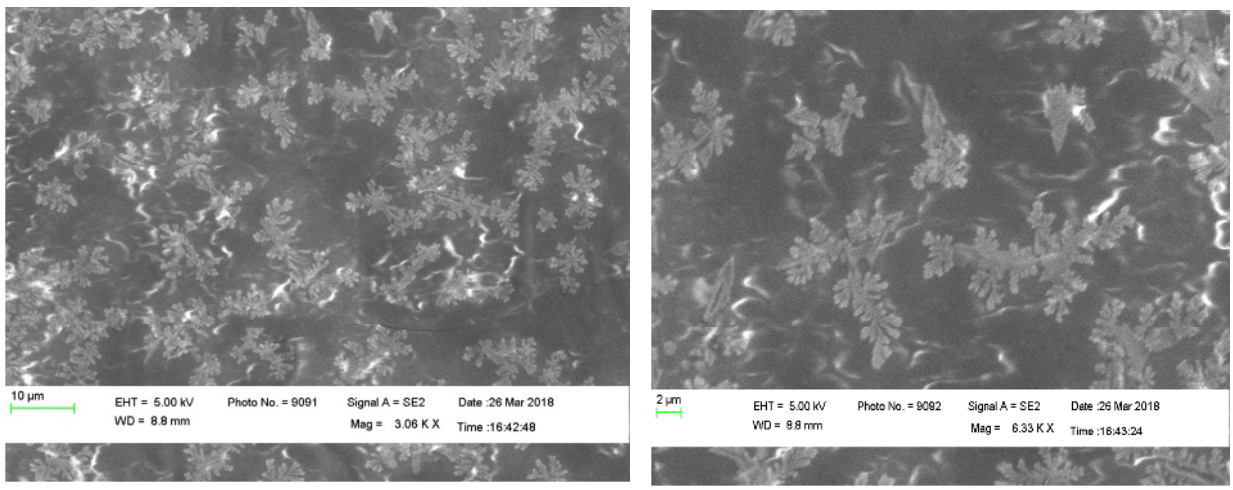


Fig4.5.b FT-IR spectrum of BF-CDs

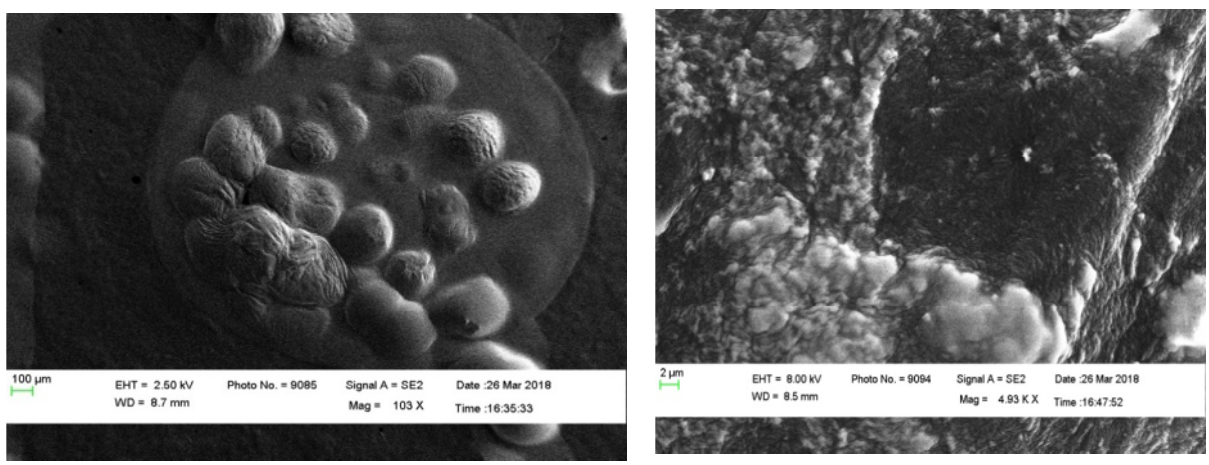
The above data indicates the presence of different functional groups like hydroxyls, carboxyl, nitrogenous groups and hydrocarbons. Thus these functional groups impart hydrophilicity to the carbon dots which inturn provided with good water solubility and hence these CDs may found applications in drug delivery system. Besides the presence of C=C stretch contributes to the fluorescence of the CDs due to the π-π^* transition

4.3.2 SEM (Scanning electron microscopy)

The SEM analysis was carried out for the as-synthesized carbon dots. The study revealed the lateral dimensions of both the CDs.



(a)



(b)

Fig.4.6 SEM images of the prepared carbon dots (a) TF-CDs (b) BF-CDs

SEM micrographs in Fig.4.6 (a) showed the condensed leaf-like structure. Fig.4.6 (b) visualized that the shapes of the prepared TF-CDs largely remained unaffected by the hydrothermal treatment.

4.3.3 3D Optical Profilometer

3D optical profilometer image analysis was carried out to obtain the surface morphology of the prepared carbon dots.

The samples for 3D optical profilometer characterization were prepared by dropping aqueous suspension (˜0.02 mg/mL) of the synthesized carbon dots on a fresh glass plate and dried under vaccum at 80˚C.

The average roughness, Ra, (the average deviation of all points roughness profile from a mean line over the evaluation length), root-mean-square roughness, Rq, (the average of the measured height deviations taken within the evaluation length and measured from the mean line) were observed.

3D optical image of TF-CDs

The synthesized carbon dots were characterized by microscopy techniques for an understanding of structural parameters. This gives us insight about the topography, roughness of the synthesized CDs. Fig 4.7.1 shows the 3D optical image of the synthesized TF-CDs.

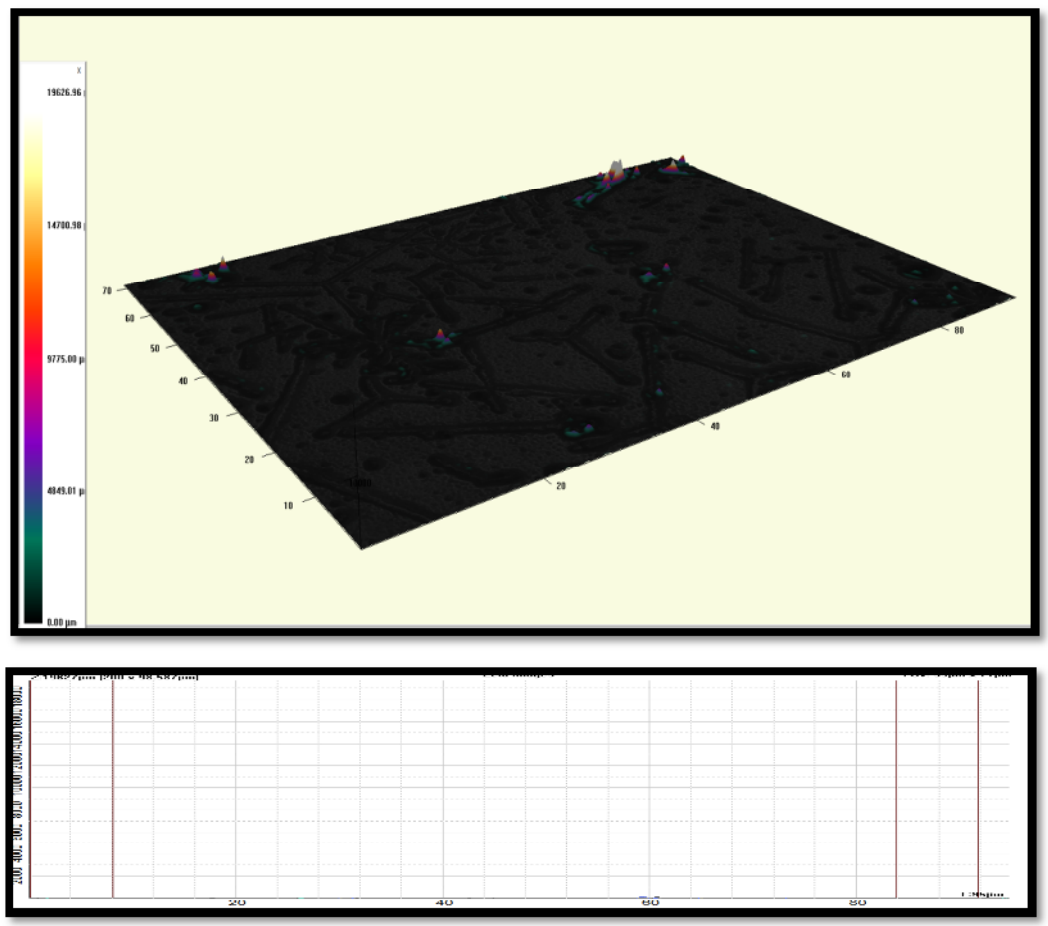


Fig.4.7.1 3D Optical image of TF-CDs and topographic image of TF-CDs

Images were clearly demonstrated the smoothness of TF-CDs with capping of phytochemicals over the surface of CDs.

***Borassus flabellifer* (fruit pod)**

To measure the size and thickness of BF-CDs 3D Optical Profilometer was used. The 3D Optical image of the synthesized carbon dots from *Borassus flabellifer* (fruit pod) were displayed in Fig 4.7.2

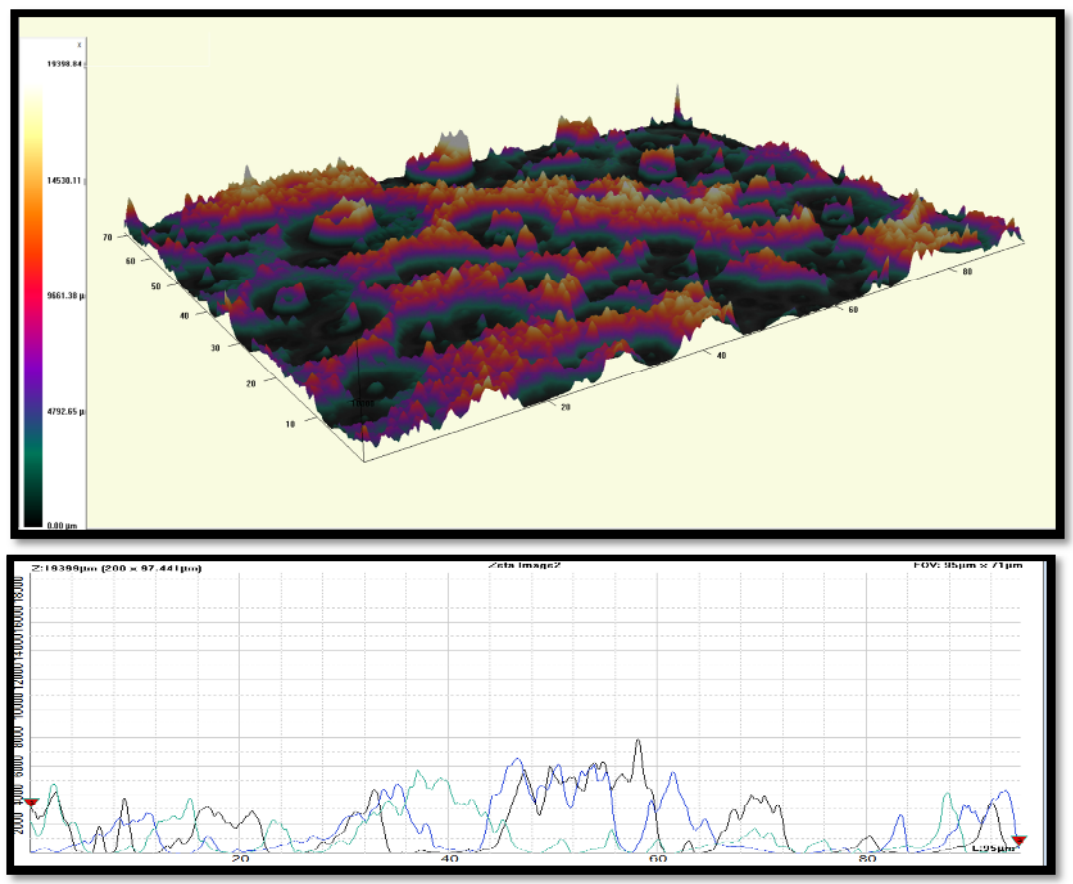


Fig 4.7.2 3D Optical image of BF-CDs and topographic image of BF-CDs

Sample	R_a	R_Q
TF-CDs	5.486	18.12
BF-CDs	1343.12	1636.2

Table.4.6. R_a and R_Q values for TF-CDs and BF-CDs

From the above data it was clear that the roughness profile for TF-CDs was found to be very smooth, while the surface roughness was too high in case of BF-CDs

4.4 Application

Antibacterial studies

Carbon nanoparticles were found to have effective inhibition against the growth of pathogens because they have the ability to absorb UV radiation and also found to have lower toxicity level.

In the present investigation, TF-CDs and BF-CDs were tested for its antibacterial activity which was performed using cultures of *Staphylococcus aureus*, *Escherichia coli,* and *B.cereus*. Agar disc diffusion technique was adopted to perform the assay.

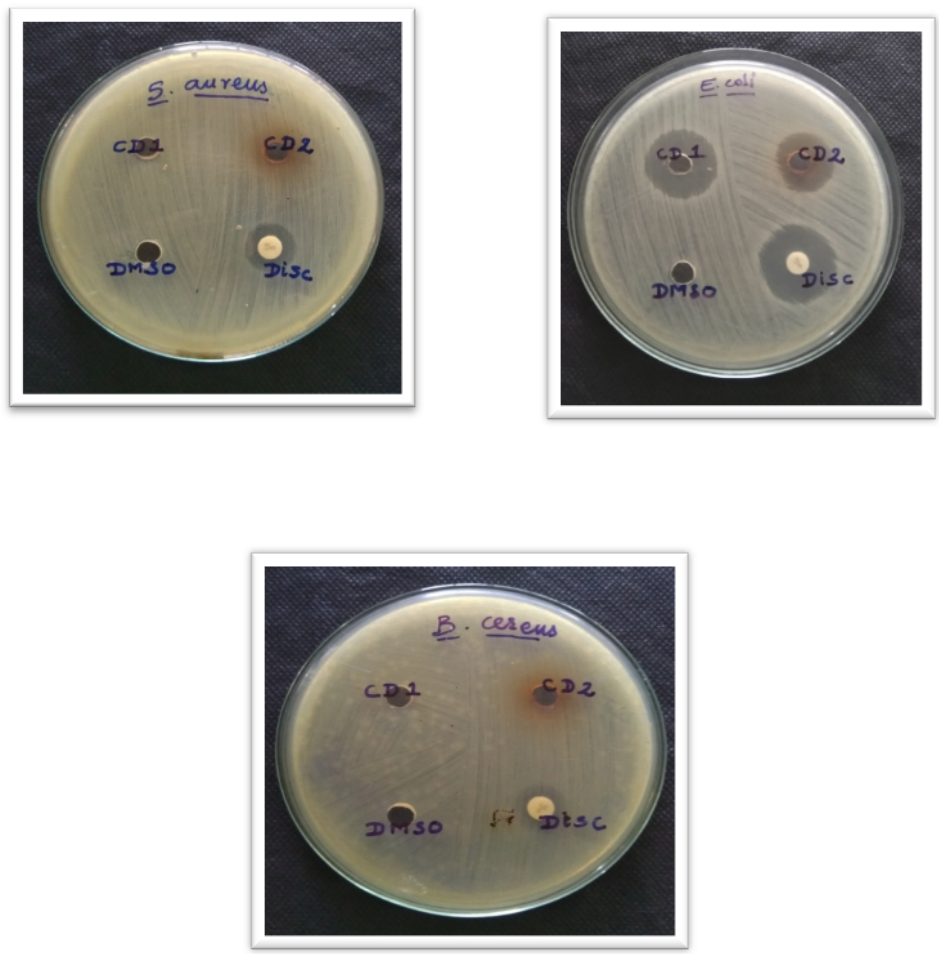


Fig.4.8. Zone inhibition of *Staphylococcus aureus*, *Escherichia coli,* and *B.cereus* on TF-CDs and BF-CDs

There was numerous reports stated that gram positive bacteria were probably extra sensitive to the nanoparticles than gram negative bacteria, so gram negative bacteria were more efficient in inhibition.

Microorganism used	Zone of inhibition in mm			
	CD 1	CD 2	DMSO	Antibiotic Disc LE 5
B.cereus	**1**	**4**	**Nil**	**3**
S.aureus	**1**	**3**	**Nil**	**3**
E.coli	**5**	**4**	**Nil**	**7**

Table.4.7 Zone of inhibition of CDs

The synthesized CDs exhibited antibacterial properties and the antibacterial studies revealed that TF-CDs (CD1) and BF-CDs (CD2) maximum inhibition of 5 mm and 4mm respectively against *Escherichia coli* in comparision with *Staphylococcus aureus*, and *B.cereus.*

The enhanced antibacterial activity of carbon nanoparticles can be attributed to their increased surface area available for interation with enhanced bacterial effect.

These carbon dots exhibited antibacterial properties due to increased surfaced area, reduced particle size and surface abrasive surface texture.

The zone of inhibition was found to be much higher than the standard disc used which exposed the need offurther engineering of nanoparticles to obtain the desirable effects.

Summary and Conclusion

In this present work, we developed a green and low cost hydrothermal method for the preparation of carbon dots using *Trigonella foenum graecumL* (stem) and *Borassus flabellifer* (fruit pod) which are water soluble and are eco-friendly. Both the precursors were biodegradable wastes and re-usability of the wastes made it cost-effective and less toxic by the use of green chemistry. The UV-Visible spectrum exhibited absorption maximum for TF-CDs at 223 nm and another peak at 307 nm which was attributed to the π - π^* transition and for BF-CDs the absorption maximum at 290 nm and 330 nm.

- The diameter of the prepared TF-CDs and BF-CDs were calculated using the method described by Hengleir & Co worker and found to be 1.22 nm & average of 1.39 nm respectively.
- The functional groups presented on both the CDs were studied using FT-IR spectrum and confirmed the presence of C=O, C=C, O-H, N-H groups.
- The prepared CDs were found to hold good photoluminescent property with maximum emission around 450nm. The PL emission might be attributed to the varied particle sizes of the CDs.
- 3D Optical images were taken for both CDs. from the Fig 4.6 it was clear that FG-CDs were smoother as compared to BF-CDs. They might also found applications in biological field since it showed maximum zone of inhibition for E.Coli bacteria.

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