SYNTHESIS OF NOVEL CARBON DOTS FROM *Polyalthia longifolia* LEAF FOR *In Vitro* CYTOTOXICITY AGAINST HELA CELLS

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by

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P. longifolia	*Polyalthia longifolia*
CQDs	Carbon quantum dots
SWNTs	Single-walled nanotubes
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CDs	Carbon dots
UV	Ultraviolet
LED	light- radiating diode
FL	Fluorescent
PDT	Photodynamic treatment
PTT	Photothermal treatment
H_2SO_4	Sulfuric acid
Na_2CO_3	Sodium carbonate
HNO_3	Nitric acid
PL	Photoluminescence
µg/mL	Microgram per milliliter
µm	Micrometre
°C	Celsius
g	Gram
rpm	Revolutions Per Minute
λ	wavelength
NaCl	Sodium Chloride
CO_2	Carbon Dioxide
θ	Theta

μL	Microlitre
Sp^2	Sp orbitals
mL	Milliliter
kV	Kilovolt
nm	Nanometer
cm^{-1}	reciprocal centimetres
mA	Magnetic absorbance
pH	Potential of Hydrogen
mg/mL	milligrams per milliliter
mg/g	Milligram per gram
SD	Standard deviation
MRI	Magnetic resonance
NIR	Near infrared light
FLIM	Fluorescence lifetime imaging microscopy
IFE	Inner filter effect
FRET	Fluorescence resonance energy transfer
PET	Photoinduced Electron Transfer
TKI	Tyrosine kinase inhibitors
GOx	Glucose oxidase
GA	Gallic acid
CA	Calcium alginate
IARC	International Agency for Research on Cancer
WHO	World Health Organization
ATCC	America Type Culture Collection
DMSO	Dimethyl sulfoxide
FBS	Fetal bovine serum
PBS	Phosphate uffered saline

XRD	X-ray diffraction
FTIR	Fourier Transform Infra-Red
KBr	Potassium Bromide
RAW 264.7	Mouse monocyte macrophage cell line
ELISA	Enzyme-linked Immunosorbent Assay
TEM	Transmission Electron Microscopy
DMEM	Dulbecco's Modified Eagle Medium
UV-Vis	UV-Visible Spectrum
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide Assay
ROS	Reactive oxygen species
EC_{50}	Half maximal effective concentration
DCFH-DA	2′,7′-dichlorodihydrofluorescein diacetate
DCFH	2′,7′-dichlorodihydrofluorescein
CCK-8	Cell Counting Kit 8
SPSS	Statistical Package For The Social Sciences
ANOVA	Analysis of variance formula

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ABSTRAK

Titik karbon (CD), yang terkenal dengan sifat pendarfluornya yang luar biasa, telah digunakan secara meluas dalam bidang perubatan dan farmasi kerana biokompatibiliti dan bersaiz nano. Walau bagaimanapun, potensi kesan sitotoksik CD yang diperoleh daripada *Polyalthia longifolia* yang kaya dengan pelbagai aktiviti farmakologi masih kurang diterokai. Untuk menangani jurang ini, kajian semasa telah dijalankan dengan mensintesis, mencirikan dan menyiasat ketoksikan terhadap sel RAW264.7, serta sitotoksisiti yang diaruh oleh cahaya terhadap sel HeLa bagi CD yang diperoleh daripada *P. longifolia*. Pelbagai kaedah, termasuk TEM, XRD, UV-vis, potensi zeta, dan spektroskopi inframerah-transformasi Fourier (FTIR) telah digunakan untuk mencirikan CD. Selain itu, ketoksikan CD yang disintesis telah dinilai terhadap sel RAW 264.7, dan sitotoksisiti yang diaruh oleh cahaya telah dinilai terhadap sel HeLa di bawah cahaya putih. Selain itu, kesan pengaruhan cahaya pada penjanaan ROS dalam sel HeLa juga disiasat. CD berbentuk sfera hitam yang disintesis mempamerkan saiz purata 6.29 ± 0.31 nm (TEM), yang terdiri daripada fasa kristal dan amorfus karbon (XRD), dengan nilai potensi zeta negatif sebanyak -20.7 ± 2.01 mV. CD yang disintesis memancarkan pendarfluor biru apabila terdedah kepada cahaya UV pada 365 nm dan mempamerkan puncak penyerapan UV yang ketara di rantau UV. Analisis FTIR mendedahkan kewujudan kumpulan berfungsi yang pelbagai pada CD. Penilaian ketoksikan CD yang disintesis menunjukkan ketoksikan yang rendah dan menggalakkan pertumbuhan sel RAW264.7 dalam julat 500-8000 μg ($P<0.05$). Tambahan pula, sitotoksisiti yang disebabkan oleh pengaruhan cahaya

mendedahkan sitotoksisiti yang bergantung kepada pengaruhan cahaya terhadap sel HeLa ($P < 0.05$). Akhirnya, penyiasatan kesan pengaruhan cahaya terhadap CD pada penjanaan ROS dalam sel HeLa mendedahkan peningkatan tahap ROS intraselular yang membawa kepada kematian sel HeLa ($P<0.05$). Keputusan kajian ini menunjukkan peningkatan sitotoksisiti akibat pengaruhan cahaya terhadap CD yang diperoleh daripada *P. longifolia*, memberikan wawasan baru dalam penngunaan CD dalam bidang kesihatan.

SYNTHESIS OF NOVEL CARBON DOTS FROM *Polyalthia longifolia* LEAF FOR *In Vitro* CYTOTOXICITY AGAINST HELA CELLS

ABSTRACT

Carbon dots (CDs), known for their outstanding fluorescence properties, have found extensive applications in medicine and pharmacy owing to their biocompatibility and nano-sized attributes. However, the potential cytotoxic effects of CDs derived from *Polyalthia longifolia* rich with various pharmacological activity remain underexplored. To address this gap, the current study synthesized, characterized, and investigated the toxicity against RAW264.7 cells, as well as photo-induced cytotoxicity against HeLa cells of the CDs derived from *P. longifolia*. Various methods, including transmission electron microscopy (TEM), X-ray diffraction (XRD), ultraviolet-visible (UV-vis) spectroscopy, zeta potential, and Fourier-transform infrared spectroscopy (FTIR), were employed to characterize the CDs. Additionally, the toxicity of the synthesized CDs was evaluated against RAW 264.7 cells, and the photo-induced cytotoxicity was assessed against HeLa cells under white light. Moreover, the impact of photo activation on ROS generation in HeLa cells also investigated. The synthesized spherical black spheres exhibited an average size of 6.29 ± 0.31 nm (TEM), consisting of both crystalline and amorphous phases of carbon (XRD), and a negative zeta potential of -20.7 ± 2.01 mV. The synthesized CDs emitted blue fluorescence when exposed to UV light at 365 nm and exhibited a prominent UV-visible absorption peak in the UV region. FTIR analysis revealed the existence of diverse functional groups on the CDs. The toxicity evaluation of synthesized CDs indicated low toxicity, promoting RAW264.7 cell viability within the 500–8000 µg range ($P<0.05$). Furthermore, the photo-induced cytotoxicity revealed photo

activation-dependent cytotoxicity against HeLa cells ($P<0.05$). Finally, the investigation of the impact of photo activation on ROS generation in HeLa cells revealed an increase ($P<0.05$) in intracellular ROS levels leading to HeLa cell death. These results demonstrate the photo-induced cytotoxicity of non-toxic CDs derived from *P. longifolia*, providing new insights into the potential healthcare applications of CDs.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Carbon mainly exists in graphite, diamond, and carbon nanotubes. In recent years, substantial research struggles have been dedicated to developing advanced carbon nanomaterials such as carbon dots due to their unique biomedical, electronic, mechanical, thermal and optical properties (Jiang *et al.,* 2014). In the early 21st century, carbon quantum dots (CQDs) were first discovered in the research for the purification of fluorescent single-walled nanotubes (SWNTs) (Xu *et al.,* 2004) whereby it was later on widely known as carbon dots (C-dots) (Jelinek, 2017). In general, C-dots are semiconductor nanoparticles with a diameter size of less than 10 nm (Singh, 2020) that are composed of graphitic carbon that are known as sp^2 hybridization (Dong, *et al.,* 2010). The luminescent properties of carbon dots allow for their applications in various fields such as bio-imaging/sensing, analytical chemistry, photocatalysis and optoelectronic devices (LEDs) (Jiang *et al.,* 2014; Zhang *et al.,* 2013; Mao *et al.,* 2014; Yan *et al.,* 2014). Compared with commonly used metal-based quantum dots, carbon dots have higher biocompatibility and lower toxicity (Huang *et al.,* 2014), suggesting they are more suitable for bio-applications. In this sense, this research was proposed to develop new nanotechnology-based products, such as novel carbon dots from the non-toxic *Polyalthia longifolia* leaf, which possesses good anti-cancer properties by using modern nanotechnological approaches.

Cancer is the invasion of neighbouring tissues that characterises the terrible and potentially fatal condition by abnormally proliferating cells, which destroy these tissues (Gennari *et al.,* 2007). Given that cervical cancer is the fourth most frequent malignancy in women worldwide and accounts for more than a quarter of a million annual fatalities, it is an important global health issue (Small *et al.,* 2017). Although cervical cancer is the leading source of cancer death globally, there has been very little progress in the effective treatment against this cancer over the past decade (Li *et al.,* 2016). Utilizing medicinal plants is an alternative approach to counteract the negative effects of synthetic medications. Many medicinal plants have been testified to display preventive and healing properties for cancer. National Cancer Institute of United States has conducted screenings about 114,000 extracts with anticancer potential (Shoeb *et al.,* 2006).

One such crucial medicinal plant is *P. longifolia* (Sonn.) Thwaites (PL) (Family: Annonaceae). *P. longifolia* is a tall evergreen tree grown in Southeast Asia, Africa, Australia, and New Zealand (Katkar *et al.,* 2010). It is often grown in tropical areas for its attractive appearance (Bose *et al.,* 1998). Macroscopically, the versatile *P. longifolia* can reach over 15.0 m high with symmetrical, pyramidal growth and weeping pendulous branches with linear-lanceolate 1 to 1.5 cm broad leaves. *In traditional medicine, P. longifolia* have long been used as a tonic for rejuvenation (Hemagirri *et al.,* 2022). The polyphenol-rich *P. longifolia* leaf methanol extract exhibited good antioxidant and hepatoprotective activities against paracetamol-induced oxidative damage in mice (Jothy *et al.,* 2012). The non-toxic *P. longifolia* leaf methanol extract was also revealed no genotoxic effect against the *onion* root tip (*Allium cepa)* assay. In addition, a single dose of *P. longifolia* leaf extract at 5000 mg/kg body weight given orally to rats was also proven safe without any apparent

toxicity (Jothy *et al.,* 2013). Additionally, *P. longifolia* methanolic extract (PLME) reduced mortality in irradiated X-ray mice as compared to the untreated group, which had 100% mortality (Jothy *et al.,* 2016). Also proven to be cytotoxic against ovarian carcinoma, breast adenocarcinoma, oral carcinoma, and cervical carcinoma cell lines in vitro is clerodane, a diterpenoid synthesized from the leaf of *P. longifolia* (Sashidharan *et al*., 2011). Along with this, *P. longifolia* leaf extract demonstrated cytotoxicity against HeLa cancer cells by causing apoptotic cell death and miRNA modulation (Vijayarathna, 2016; Vijayarathna *et al.,* 2017a; Vijayarathna *et al.,* 2017b). The most recent study conducted by our team also reported the preclinical antitumor activity *of PLME* in the HeLa cells tumour xenografted animal model (Cilwyn-Shalitha, 2022). Even though the latest studies have indicated that *P. longifolia* leaf extract can inhibit the growth of tumour xenografts established from HeLa cells in a nude mice model, there are no new nanotechnology-based products such as novel carbon dot developed from non-toxic agent *P. longifolia* leaf as a final product from medicinal plants as anticancer agents against cervical cancer.

1.2 Problem Statement

Over countless years, home towners have developed a close relationship with medicinal plants as a source of potential treatments for various illnesses. The development of anticancer drugs using medicinal plants has gained attention recently and the fact that various compound presence in the plants could exhibits cytotoxic effect against cancer cells. The non-toxic *P. longifolia* showed effective *in vitro* and *in vivo* anticancer activity against cancer cell lines by modulating several miRNA and inducing apoptotic cell death, as was described in Introduction. There has been a lot of interest in the recent development of anticancer medicines derived from

medicinal plants. However, seldom a plant extract itself can be used as a final product. In order to determine whether these extracts will undoubtedly be the source of anticancer activity or whether they may be used to achieve cancer blocking or restorative effects in the human body, attention must be focused on this evaluation. Subject to the method of extraction, plant extracts can contain an enormous variety of phytochemicals. In many cases, from a pharmacological point of view, it is interesting to work with crude extract or fractions instead of a single isolated compound. This could be due to multi-targeting effect of the various phytochemicals presence in the plant extract. In any case, lack of knowledge of the active compounds, synergistic effect of the extract compounds, poor stability, solvent toxicity, and low solubility of the bioactive compound must be overcome in order to achieve a final product or developed new products from medicinal plants. Interestingly, recently many nanotechnology-based strategies have been proposed as an alternative to solve these problems. In this sense, the present work was conducted to developed new

nanotechnology-based products such as novel carbon dot from anticancer agent *P. longifolia* leaf by using nanotechnological approaches.

1.3 Research aim and objectives

This research was conducted to synthesize novel carbon dots from *P. longifolia* Leaf extract for *in vitro* cytotoxicity against HeLa cells.

1.4 Specific Objectives

In this work, the objectives are:

1. To synthesis the novel carbon dots (CDs) from *P. longifolia* leaves.

2. To characterize the CDs synthesized from *P. longifolia* leaves.

3. To evaluate the toxicity of the synthesized CDs from *P. longifolia* leaf against the mouse monocyte macrophage (RAW 264.7) cells and photo-induced cytotoxicity against HeLa cells.

CHAPTER 2

LITERATURE REVIEW

2.1 Polyalthia longifolia

Polyalthia longifolia is called bogus Ashoka, Buddha Tree, and Indian Fir tree, which originated in Sri Lanka, India and widely distributed to many Asian countries, especially in Malaysia (Jothy *et al.,* 2013). *Polyalthia longifolia* falls under the kingdom Plantae, which encompasses all living organisms that are classified as plants. Within the kingdom Plantae, *P. longifolia* belongs to the phylum Magnoliophyta, commonly referred to as the flowering plants. *P. longifolia* is classified under the class Magnoliopsida, also known as the dicots. Dicots are a diverse group of flowering plants that possess two cotyledons (embryonic leaves) in their seedlings. The order Magnoliales encompasses various families of flowering plants, including the family Annonaceae to which this plant belongs. *P.longifolia* is a member of the family Annonaceae, commonly referred to as the custard apple family. Many plants in this family are renowned for their medicinal properties. The genus name "*Polyalthia*" is derived from the Greek word "poly" meaning "many" and "althos" meaning "nourishment," possibly referring to the nutritional value of the plants in this genus. The species name "*longifolia*" is derived from the Latin words "longus" meaning "long" and "folium" meaning "leaf," referring to the long, lanceolate leaves characteristic of *P. longifolia* (Wallis, 1985). *P. longifolia* with its taxonomic classification and therapeutic benefits, represents a promising plant species in the field of traditional medicine and modern healthcare.

2.1.1 Therapeutic benefits of *P. longifolia*

The significant therapeutic plant with a different pharmacological range that includes antioxidant, anti-cancer, anti-ulcer, anti-inflammatory, and anti-microbial properties make it a subject of interest for further exploration (Yadav and Sardesai, 2000). *P. longifolia* has been used in traditional medicine for centuries (Dixit *et al.,* 2014). The plant has been used to treat various ailments such as fever, diarrhea, dysentery, and wounds (Kuo *et al.,* 2002). The plant has also been used to treat skin diseases, rheumatism, and respiratory problems (Saleem *et al.,* 2005). The plant has been found to possess anti-inflammatory, analgesic, and antimicrobial properties, which have been attributed to its traditional use. Flavonoids such as Rutin, Chrysin and Proanthocyanidin presented on ethanolic leaf extraction of *P. longifolia* showed anti-cancer, anti-microbial and antioxidant properties according to Zhang in 2015. It is utilized as conventional medication for different natural planning utilized for treating duodenal ulcers with 89.1% inhibition rate (Malairajan *et al.,* 2008). These substances neutralize free radicals, lowering oxidative stress and defending cells against harm. The plant's antioxidant action may be extremely important in preventing several chronic diseases, such as cancer and certain forms of cardiovascular and neurological disorders (Zhang *et al.,* 2015; Cheng *et al.,* 2017). Extracts of *P. longifolia* have demonstrated strong antibacterial effects against a variety of bacterial species. The ethanol extract of *P. longifolia* leaves was found to have substantial inhibitory effects against *Staphylococcus aureus,* a widespread bacterium that can cause skin infections and respiratory tract infections, in a study conducted by researchers (Savu *et al.,* 2022). Additionally, another study examined the anti-bacterial capabilities of *P. longifolia* root extracts and found that they were efficient against *Escherichia coli*, the bacterium that causes gastroenteritis and urinary tract infections (Chanda *et al.,* 2010). *P.*

longifolia leaves affirmed its antifungal development against *Cryptococcus neoformans* (a human microbe), *Neurospora crassa* (saprophyte) and *Candida albicans* (Bhattacharya *et al.,* 2015). *P. longifolia* re-established cancer prevention agent status, further developed lipid profiles, and weakened the sores in the tissues protects against Cd-actuated kidney poison levels by proposing good antioxidant properties. *P. longifolia* has anti-plasmodial exercises obtained the IC_{50} values at 9.5 µg/mL have great *in vitro* movement against human malaria (Kwansa et al., 2019). Recent studies have explored the impact of *P. longifolia* extracts on cancer cell migration and invasion. For instance, Chen *et al.* in 2021 examined the effects of *P. longifolia* leaf extract on human lung cancer cells. The extract exhibited significant inhibitory effects on A549 cell line migration and invasion, suggesting its potential to impede the metastatic processes of lung cancer (Chen *et al.,* 20121). Studies conducted previously showed extraction of *P. longifolia* leaf exhibit anti-cancer activity by inhibiting cell viability on colon cancer cells and HeLa cells through MTT assay with proportional IC_{50} value of 6.1 to 22 µg/mL (Vijayarathna *et al.,* 2017). *P. longifolia* extract has been found to inhibit the oxidation of lipids in the liver and reduce the level of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) that are associated with liver damage. The plant extract has also been found to possess DNA protective properties and can inhibit the formation of DNA adducts (Jothy *et al.,* 2012). Accordingly, these present literature searches provided great ideas for the therapeutic potentialities of the use of as *P. longifolia* resources in the development of more promising remedial agents.

2.2 Carbon dots (CDs)

Carbon-based nanomaterials draw expanding consideration because of the undeniable power in regard of environment friendly drawn in significant consideration since detailed research (Xu *et al.,* 2004). The discovery of fluorescent carbon nanoparticles in 2006 marked a significant advancement in the research on the properties of nanomaterials. Sun *et al.* (2006) later gave these nanoparticles the moniker "carbon quantum dots" and developed a synthetic technique to create CQDs with significantly increased fluorescence generation (Lim *et al.,* 2015). CDs that consist of several layers of “zero-dimensional” graphene sheets with sizes between 1 nm to 10 nm which exhibit strong quantum confinement effect whereby their band gaps strongly rely on their sizes (Hang *et al.,* 2021).

Owing to the prevalent optical traits such as photoluminescence, magnificent light security, obstruction to light bleaching, low toxicity, climate friendly and great biocompatibility. These nanoparticles emit vivid and colorful light when they are stimulated by light of particular wavelengths. Controlling the size, chemical structure of the surface, and composition of carbon dots allows for precise tuning of their fluorescence emission (Liu *et al.,* 2020). Their usage in bioimaging, sensing, and optoelectronic devices has been made possible by this unique optical characteristic (Azam *et al.,* 2021). Easy preparation mass of CDs has been combined and they show thrilling benefit in different application fields, including biomedicine, sensing catalysis light- radiating diode (LED), and photovoltaic devices and so on (Xia *et al.,* 2019). A crucial aspect in increasing the photoluminescence in CDs is surface passivation. Surface passivation involves treating CDs with different functional

groups, which produces surface flaws (Jaiswal *et al.,* 2021; Sharma *et al.,* 2019). A study conducted by Radhakrishnan and colleagues hence proved that self-passivated CDs from ivy gourd along glycine and L-cysteine displayed high fluorescence in Cu^{2+} and Pb^{2+} ion detection (Chandrasekaran and Sethuraman, 2019). CDs have unique electronic and optical properties due to their small size, which allows them to spatially confine charge carriers, leading to a high quantum yield of light emission. These characteristics make carbon dots suitable for various biomedical applications. CDs have become an intriguing class of nanomaterials with outstanding optical qualities and a wide range of uses. They exhibit low toxicity and can be easily functionalized to enhance their biocompatibility further (Wang *et al.,* 2022). This property enables their utilization as fluorescent (FL) probes for bioimaging, drug delivery systems, and bio-sensing platforms (Azam *et al.,* 2021). In vitro imaging is performed to give bountiful data on the tomography skill, conveyance and chemotherapeutic of tests in cells (Nocito *et al.,* 2021). CDs were effectively utilized in an assortment of cell infection by transformation imaging, for example, human brain undeveloped cells and HeLa cells. Whereas during drug delivery by 3 hours of transfection, CDs-DNA compounds were able to penetrate the cells as they continued to exhibit their FL feature while being transported across different wavelength stimulation (Tuerhong *et al.,* 2017). Moreover, Jia and co-workers investigated the utilization of CDs disconnected from *Hypocrella bambusae* as photothermal treatment (PTT) and photodynamic treatment (PDT) in malignant growth treatment (Jia *et al.,* 2018). These current publications give good potential to the possible utilization of CDs blended from raw items as a helpful specialist that can use to determine worldwide wellbeing concerns, particularly irresistible illness, and disease such as cancer.

2.2.1 Mechanism of Photoluminescence in Carbon Dots

The photoluminescence (PL) mechanism in carbon dots (CDs) involves the emission of light upon excitation by photons (Zhang *et al.,* 2021). One of the widely discussed mechanisms is the surface state model, which suggests that the surface functional groups or defects in CDs play a crucial role in photoluminescence (Xia *et al.,* 2018; Liu *et al.,* 2019). Excitation of CDs by photons leads to electron promotion from the valence band to these energy states. Subsequent relaxation of the excited electrons back to the valence band results in the emission of light (Ai *et al.,* 2021). Surface passivation and functionalization can affect the density and energy distribution of these surface states, influencing the photoluminescence properties (Sun *et al.,* 2006). The quantum confinement effect is another mechanism proposed to explain the photoluminescence in CDs (Guo *et al.,* 2016). Due to their small size and high surface-to-volume ratio, CDs exhibit quantum confinement effects on their electronic structure (Dong *et al.,* 2017). Haldar *et al.,* (2018) successfully reported defect induced range of photoluminescence from MoS_2 CDs that is mostly used as bio-imaging labelling. The confinement of electrons and holes within the nanoscale dimensions of CDs enhances radiative recombination, leading to efficient light emission. CDs derived from citric acid and diaminonaphthalene via solvothermal method produced multicolor FL like blue (1.95 nm), green (2.41 nm), and orange (4.90 nm) colors with different CDs sizes respectively (Yuan *et al.,* 2018). The presence of surface states creates energy levels within the bandgap of CDs (Herbani *et al.,* 2018). The specific energy levels and electronic transitions influenced by quantum confinement contribute to the observed photoluminescence properties. Recent studies have highlighted the significance of defect-induced emissions in CDs. Structural defects, such as carbon vacancies or heteroatom doping, can introduce localized

energy states within the CDs (Li *et al.,* 2020; Zhang *et al.,* 2020). These defects act as trapping sites for charge carriers, facilitating radiative recombination and light emission (Demchenko *et al.,* 2016). The nature and density of defects, as well as the impact on the photoluminescence properties, have been investigated in-depth. The energy level alignment within CDs also plays a crucial role in their photoluminescence. The presence of energy levels that facilitate efficient charge transfer and radiative recombination influences the emission properties. The energy levels can be influenced by the carbonization temperature, precursor composition, and surface functionalization (Kwon *et al.,* 2015). The interplay between these factors determines the fluorescence properties of CDs. This provides insights into the origin and control of photoluminescence properties, aiding in the design and optimization of CDs for various applications. This knowledge will enable the design and optimization of carbon dots with enhanced fluorescence efficiency and stability, advancing their potential in bioimaging, sensing, optoelectronics, and other fields.

2.2.2 Synthesis of CDs from natural resources

The "top-down" and "bottom-up" methodologies are two distinct ways that can be broadly categorized as part of the synthesis of CDs. These methods offer adaptable methods to design CDs with the appropriate characteristics for certain applications. CDs synthesized from natural resources are generally displayed as better compared to chemically derived CDs (Zulfajri *et al.,* 2020). Green synthesis methods have gained prominence due to their cost-effectiveness and reduced environmental impact compared to traditional physical and chemical processes (Aswathi *et al.,* 2023). Moreover, these methods offer the opportunity to convert natural materials into valuable products, contributing to the efficient management of resources and

addressing environmental concerns. Sustainable synthesis techniques prioritize using solvents and reagents that are safe for the environment, generating relatively little hazardous waste, and lowering their carbon footprint (Ouyang *et al.,* 2023). Utilizing easily available and inexpensive precursors, green synthesis techniques for CDs frequently lower the overall production costs. **Table 2.3** summarizes the advantages and drawbacks of both synthesis approaches associated to CDs properties. Synthesizing novel carbon dots via simple carbonization which involved thermal decomposition of the *P. longifolia* leaf was studied.

2.2.2 (a) Top-down Approaches

The top-down approach involves the fabrication of CDs through the modification and manipulation of pre-existing carbon-based materials. This method typically starts with a bulk carbon source, such as carbon nanotubes, graphene, or carbon black, which is then subjected to mechanical, chemical, or electrochemical processes to generate CDs with desired properties (Wang and Hu, 2014). For example, mechanical exfoliation of graphite or carbon nanotubes can produce CDs by breaking down the larger structures into smaller nanoparticles. Chemical oxidation and ultrasonic treatment can also be employed to obtain CDs with tailored surface properties. Chemical oxidation refers to a process of chemical reaction with a common oxidizing agents like nitric acid (HNO_3) or sulfuric acid (H_2SO_4) to produce CDs. Usage of oxidizing agent helps in breaking down of carbonaceous compounds in the raw materials that will followingly form various functional groups which leads to contribution in surface passivation. For instance, tea residue and mahogany fruit shell was used as carbon precursors where it is subjected to chemical oxidation process with 50 mL of HNO_3 and H_2SO_4 followed by neutralization using sodium carbonate

(Na_2CO_3) process (Gunjal *et al.,* 2019). Besides that, ultrasonic treatment involves subjecting precursor solution to high-frequency sound waves (acoustic cavitation), an alternation of pressure regions within the solution. The work of Zaib *et al.,* (2020) witnessed ultrasonic treatment of CDs using *Elettaria cardamomum*. The cardamom was grinded in fine powder and dissolved in ethanol before exposed to 20 kHz high-frequency sound waves (Zaib *et al.,* 2020). Indeed, this method faces a significant challenge related to uneven heating of raw materials due to thermal effect that can impact efficiency of reaction compared to directing heating. However, integrating ultrasonic treatment with additional methods such as microwave irradiation or hydrothermal will synergistically improve the performance of CDs (Lin *et al.,* 2021). *Ficus johannis* fruit derived CDs was synthesized first via ultrasonic treatment for 15 followed by microwave-assisted under different microwave frequency power at 90 W and 270 W to produce 3.7 nm average size CDs with better biological effect (Alvand *et al.,* 2019). The top-down approach offers control over the size, structure, and surface functionality of CDs, making it a powerful method for producing customized nanomaterials (Sharma and Das 2019).

Table 2.1: The overview of CDs synthesis from different carbon precursors through "top-down" approaches.

Materials	**Process**	**Production conditions**	**Application**	**Reference**
Tea residue	Chemical oxidation	HNO_3 / 6 h	Cell-imaging	Gunjal *et al.,* 2019
Sugarcane bagasse	Chemical oxidation	H_2O_2 / 3-4 h	Fluorescence sensor	Boruah *et al.,* 2020
Green tea leaf	Chemical oxidation	H_2SO_4 / 20 h	Fluorescence sensor	Hu *et al.,* 2020
Musk melon	Chemical oxidation	H_2SO_4, H_3PO_4 / 30 min	Biosensor	Mittal *et al.,* 2019
Garlic peels, Taro	Chemical oxidation	H_2O_2 / 3-4 h	Fluorescence sensor	Boruah *et al.,* 2020
Mahogany fruit	Chemical oxidation	H_2SO_4 / 15 min	Fluorescence sensor	Gunjal *et al.,* 2019
Tomato	Chemical oxidation	H_3PO_4 / 25 min	Bio-imaging	Kailasa *et al.,* 2019
Potato	Ultrasonic treatment	3-4 h / 40 kHz	Fluorescence sensor	Kaur and Verma, 2022
Elettaria cardamomum	Ultrasonic treatment	20 kHz / 45 min	Fluorescence sensor	Zaib *et al.,* 2020

2.2.2 (b) Bottom-up Approaches

The bottom-up approach involves the synthesis of CDs from molecular precursors, building up their structure's atom by atom or molecule by molecule. The synthesis techniques for carbon nanodots included hydrothermal, ultrasonic, microwave, carbonization, and pyrolysis process categorized as bottom-up method. The precursors can include small organic molecules, sugars, amino acids, or polymers, which are subjected to controlled heating or chemical reactions to induce carbonization and the formation of CDs (Lu *et al.,* 2012). Carbonization involves the controlled thermal decomposition of organic materials to produce carbonaceous substances and several studies have focused on using sustainable resources to synthesis green CDs including peanut, lemon, oil palm and others that is listed in **Table 2.2.** A study conducted by Dager *et al.* 2019 revealed that carbonization of fennel seeds at a constant temperature at 500 °C for 3 hours, followed by 5 minutes of sonification, centrifugation and dialysis to achieve purified CDs solution. This approach resulted in good photostability due to their mono-dispersed structure (Dager *et al.,* 2019). Pyrolysis method by utilizing affordable precursors and avoiding the need for complex equipment or specialized reagents, bottom-up strategies enable cost-efficient production of CDs on a larger scale. Watermelon peel derived CDs under 220°C pyrolysis (low-temperature carbonization) obtained yellow CDs solution under 365 UV light radiation (Zhou *et al.,* 2012). The straightforward procedures enable researchers to synthesize CDs with relative ease and efficiency, facilitating their widespread adoption in both academic and industrial settings. One-step hydrothermal strategy for CDs synthesis is well-known as they are characterized by their simplicity and ease of implementation that involves reaction of carbon precursors like kiwi, turmeric, sweet potato, bamboo, rose, ginger, and cauliflower in high pressured

aqueous environment (Ahmadian, Salavati Arul and Sethuraman, 2018; Liu *et al.,* 2019; Shekarbeygi *et al.,* 2020; Liu *et al.,* 2015; Li *et al.,* 2014; Ashrafi *et al.,* 2020). Medicinal plant such as Henna leaf were used to fabricate water-soluble CDs through heating process in Teflon-line stainless steel autoclave, where the henna leaf powder was mixed with deionized water and placed in a sealed container at 180°C for 12 hours that can be stored up to 10 months. The CDs was utilized as label-free FL probe to detect specific chemotherapeutic drug named Methotrexate via human plasma serum as the stability of CDs displays higher FL intensities (Shahshahanipour *et al.,* 2019). This approach allows precise control over the size, composition, and optical properties of the synthesized CDs by adjusting reaction conditions and precursor composition (Azeem *et al.,* 2020). Also, microwave irradiation to facilitate chemical reactions and properties of CDs using microwave energy is favored by researchers as it enhances reaction efficiency by controlling heating pattern. Genc *et al.* 2020 confirms that a prepared solution of *Gingko biloba* was exposed to 500W microwave for 10 minutes. The mixture was then centrifuged and filtered using syringe filter before freeze drying (Genc *et al.,* 2020). Nevertheless, heat produced by molecule friction against an electromagnetic radiation from 1 mm to 1m range is correlated with dielectric constant that will arise fabrication limitations (Liu *et al.,* 2014). Although this method sets a drawback, it offers temporary and fast heating to synthesis good quality CDs.

Table 2.2: The overview of CDs synthesis from different carbon precursors through "bottom-up" approaches.

Materials	**Process**	**Production Conditions**	**Application**	**Reference**
Peanut	Carbonization /Pyrolysis	220 ℃ / 2 h	Fluorescence sensor	Ma *et al.,* 2017
Mangosteen pulp	Carbonization /Pyrolysis	200 ℃ / 30 min	Fluorescence sensor	Yang *et al.,* 2017
Citrus peel	Carbonization /Pyrolysis	180 ℃ / 2 h	Fluorescence sensor	Chatzimitakos *et al.,* 2017
Gynostemma	Carbonization /Pyrolysis	400 ℃ / 4 h	Fluorescence sensor	Wei *et al.,* 2019
Jinhua bergamot	Carbonization /Pyrolysis	300 ℃ / 5 h	Cell-imaging	Yu *et al.,* 2015
Prunus mume	Carbonization /Pyrolysis	300 ℃	Cell-imaging	Atchudan *et al.,* 2016
Borassus flabellifer	Carbonization /Pyrolysis	300 ℃ / 2 h	Fluorescence sensor	Murugan *et al,* 2019
Oil Palm Empty Fruit	Carbonization /Pyrolysis	250 – 400 ℃	N/A	Marpongahtun *et al.,* 2018
Lemon juice	Carbonization /Pyrolysis	100 ℃ / 45 min	Fluorescence sensor	Schneider *et al.,* 2019
Walnut shell	Carbonization /Pyrolysis	250 ℃ / 25 min	Bio-imaging (cancer cell)	Cheng et al., 2017
Fennel seed	Carbonization /Pyrolysis	500 ℃ / 3 h	Bio-imaging	Dager et al., 2019
Sugar cane molasses	Carbonization	250 ℃ / 12 h	Bio-imaging	Huang et al., 2017
Watermelon peel	Carbonization	220 ℃ / 2 h	Bio-imaging	Zhou *et al.,* 2012
Coffee (seeds)	Carbonization /Pyrolysis	220 ℃ / 20 h	Bio-imaging	Hong and Yang, 2021
Lychee	Carbonization /Pyrolysis	200 ℃ / 5 h	Bio-imaging	Sahoo *et al.,* 2020
Turmeric, Lemon & Grapefruit extract	Hydrothermal treatment	180 ℃ / 6 h	Sensor	Ahmadian,Salavati Niasari and Ghanbari, 2018
Kiwi extract	Hydrothermal treatment	180 ℃ / 12 h	Catalysis	Arul and Sethuraman, 2018
Sweet potato peels	Hydrothermal treatment	200 ℃ / 3 h	Photoluminescence sensor	Liu *et al.,* 2019
Lychii Fructus	Hydrothermal treatment	200 ℃ / 5 h	Photoluminescence sensor	Sun *et al.,* 2017
Coriander leaves	Hydrothermal treatment	240 ℃ / 4 h	Bio-imaging, bio-sensing	Sachdev and Gopinath, 2015

			detector	
Lemon juice	Hydrothermal treatment	200 ℃ / 3 h	Plant cell imaging	He *et al.,* 2018
Henna	Hydrothermal treatment	180 ℃ / 2 h	Cell-imaging	Shahshahanipour et al., 2019
Banana peel	Hydrothermal treatment	200 ℃ / 4 h	Fluorescence sensor and ink	Atchudan *et al.,* 2020
Flowers of *Osmanthus fragrans* Lour	Hydrothermal treatment	240 ℃ / 5 h	Photoluminescence sensor, cell-imaging	Wang *et al.,* 2019
Rose	Hydrothermal treatment	200 ℃ / 2 h	Fluorescence sensor	Shekarbeygi *et al.,* 2020
Bamboo leaf	Hydrothermal treatment	180 ℃ / 3 h	Fluorescence sensor	Liu *et al.,* 2015
Panax notaginseng	Hydrothermal treatment	180 ℃ / 2 h	Fluorescence sensor and bio-imaging	Zheng *et al.,* 2021
Ginger	Hydrothermal treatment	160-200 ℃ / 10-15 h	Cell-imaging	Li *et al.,* 2014
Coix seed	Hydrothermal treatment	180 ℃ / 4 h	Fluorescence sensor	Zhang *et al.,* 2021
Water hyacinths leaf	Hydrothermal treatment	200 ℃ / 4 h	Photocatalysis	Hak *et al.,* 2020
Cauliflower	Hydrothermal treatment	120 ℃ / 5 h	Fluorescence sensor	Ashrafi *et al.,* 2020
Mustard seeds	Hydrothermal treatment	200 ℃ / 12 h	Fluorescence sensor	Zhang *et al.,* 2020
Date kernel	Hydrothermal treatment	200 ℃ / 8 h	Cell-imaging	Amin *et al.,* 2018
Aloe vera	Microwave irradiation	800 W / 4-8 min	Photocatalysis, cancer cell-imaging	Malavika *et al.,* 2021
Fenugreek seeds	Microwave irradiation	500 W / 70 ℃ / 5 min	Fluorescent	Dager *et al.,* 2020
Gingko biloba	Microwave irradiation	400 – 800 W / 10 min	Photocatalysis	Genc et al., 2020
Beetroot	Microwave irradiation	350 W / 20 min	Fluorescence sensor	Praseetha et al., 2021
Potato	Microwave irradiation	250 W / 2-14 min	Photoluminescence sensor	Meng *et al.,* 2019
Sesame seed	Microwave irradiation	800 W / 10-15 min	Cell-imaging	Roshni and Divya, 2017
Lotus root	Microwave irradiation	800 W / 6 min	Cell-imaging	Gu *et al.,* 2016
Papaya seeds	Microwave irradiation	800 W / 5 min	Fluorescent sensor	Wang *et al.,* 2016

Table 2.3: Advantages and drawbacks of CDs synthesis.

Advantages	Synthesis method	Drawbacks	Reference
Allow incorporation of functional groups and surface modifications for specific applications. Can produce CDs in large quantities for commercialization.	Chemical oxidation	Complex methods that require specific reagents. Produce CDs with impurities and high residual reactants. Produce wide size distribution of CDs which is difficult to optimize properties.	Karfa et al., 2018; Singer et al., 2018; Liu et al., 2019
Allow control of CDs morphology with tailored properties. Does not involve hazardous chemicals.	Ultrasonic treatment	Specialized equipment needed for generating ultrasonic waves. Long exposure of ultrasonic leads to CDs degradation.	Deng et al., 2014; Farshbaf et al., 2018
Cost-effective and simple techniques. Produce CDs with good crystallinity. Promote sustainable approach to CDs Parameters can be optimized to control properties of CDs.	Carbonization/pyrolysis	Requires high temperature and energy consumption. Long reaction time needed for complete decomposition.	Tuerhong et al., 2017; Wang et al., 2019
Eco-friendly method by using water. Provide complete conversion of resources to CDs. Precise control over size and morphology.	Hydrothermal treatment	Limited scalability. Requires high temperature and pressure. Time-consuming procedure.	Jhonsi et al., 2018; Roman et al., 2018
Rapid synthesis of CDs. Provides uniform heating throughout reactive mixture. Enhances yield and purity of CDs	Microwave irradiation	Require high costing equipment and specific reactors	Shen et al., 2017; Farshbaf et al., 2018

2.3 Application of CDs

CDs have emerged as versatile nanomaterial with numerous applications across various fields as shown in **Figure 2.1** that emphasizes in nanomedicine (Zhang *et al.,* 2016), bioimaging (Mehta *et al.,* 2014), and fluorescence, or chemical sensing (Zhao *et al.,* 2014), in energy conservation (Wei *et al.,* 2020) and other emerging fields due to its remarkable properties.

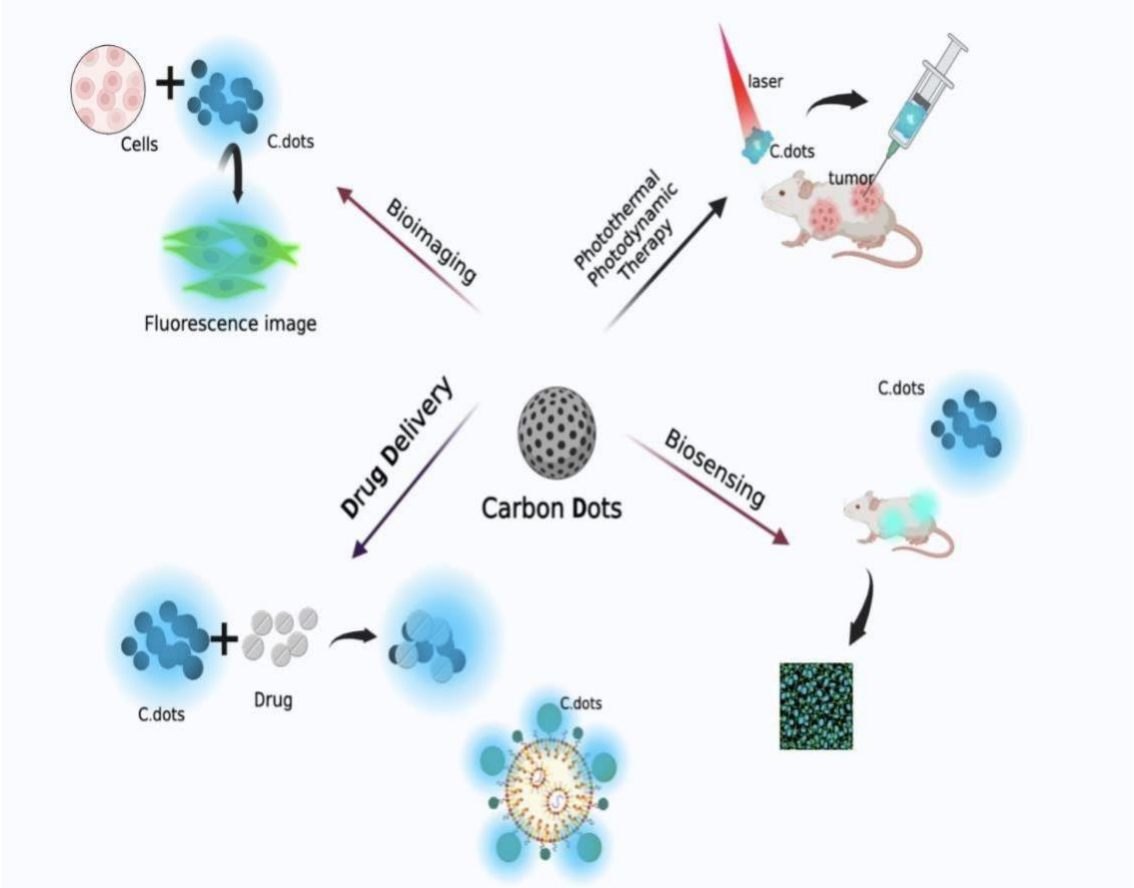

Figure 2.1: Application of CDs in various fields.

2.3.1 Carbon Dots in Cancer Studies

Since CDs have been recognized as an intriguing essence of nanotechnology, it has become a promising factor for an ideal candidate for various cancer research. The nanoscale CDs exhibits unique properties that makes it attractive for cancer studies. By involving the integration of diagnosis and therapy onto a nanoplatform offers range of benefits in cancer treatment. Nanotheranostics can enhance simultaneous monitoring of diseases and targeted delivery of therapeutic agents at one go for clinical applications. CDs shows great potential in fluorescence, photoacoustic and magnetic resonance (MRI) probes as a diagnosis and also been utilized in a photo-induced and multimodal cancer treatment (Zhou *et al.,* 2019: Chen *et al.,* 2021).

2.3.1(a) CDs in Cancer Diagnosis

In comparison with well-known CDs applications like bioimaging, sensing and nanomedicine, there is still room to understand the intracellular activity and mechanism of the potential anticancer activity of CDs. As an example, CDs prepared from oyster mushroom by hydrothermal method showed excellent anticancer activity as the synthesized CDs showed significant inhibition effects on the growth of breast cancer cells in comparison with non-cancerous human embryonic kidney cells in MTT cell viability assay (Boobalan *et al.,* 2020). This further proved that CDs showed cytotoxic effects on cancer cells and showed low toxicity or non-toxic to normal mammalian cells. Lu and co-workers found that the CDs synthesized from gallic acid (GA) can be used as an antitumor agent. The GA-CDs exhibit remarkable antitumor activity towards HeLa cells in a dose-dependent manner when performing MTT cytotoxic analysis (Lu *et al.,* 2019).

2.3.1(b) CDs in Cancer Therapy

CDs have shown promising potential in targeted drug, gene delivery, photodynamic and photothermal therapy in context of cancer theranotics (Ge *et al.,* 2014; Bhattacharya *et al.,* 2023). CDs as nanocarriers improve biocompatibility of chemotherapy drugs that reduces adverse reactions and provide safety profile of the drugs. By designing CDs incorporated stimuli-responsive components, regulation of drugs can be controlled by minimizing unnecessary exposure to healthy tissues (Calabrese *et al.,* 2020). CDs also enable precise modulation of gene expression within cancer cells for personalized treatment (Chan *et al.,* 2021).

2.3.2 CDs Application in Nanomedicine

CDs possess photothermal and photodynamic properties that can be utilized in cancer therapy. Photodynamic therapy (PDT) is a widely used clinical treatment for superficial tumors as a standalone treatment or combination with surgery. Under specific excitation, CDs can generate heat or produce reactive oxygen species, leading to targeted cell destruction in photothermal therapy or photodynamic therapy, respectively (Lan *et al.,* 2018). *Camellia japonica* flowers were utilized to synthesize CDs exhibited excellent photothermal effects with robust stability, notably at an optimal low dose of 45 μg mL^{-1} with a significant high conversion efficiency photothermal therapy (PTT) performance of 55.4% (Kim *et al.,* 2018). The CDs served as effective carriers for curcumin, facilitating its targeted delivery. Under the specific dual-wavelength irradiation, the CDs complexes exhibited a remarkable ability to generate ROS and combated bactericidal actions against *Escherichia coli* and *Staphylococcus aureus* (Venkateswarlu *et al.,* 2021). These approaches offer non-