Green synthesis and Characterization of Phyto -Metallic Quantum Dots

Dissertation submitted in partial fulfillment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

by

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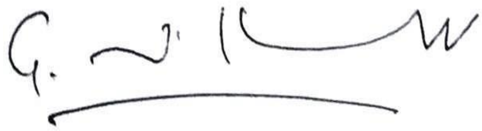
DEPT. OF BIOTECHNOLOGY AND BIOINFORMATICS

HP-173234, INDIA, May 2021

SUPERVISOR’S CERTIFICATE

This is to certify that the work reported in the B. Tech. thesis entitled **“Green synthesis and Characterization of Phyto -Metallic Quantum Dots“,** submitted by Ananyaa Kaul(171801) and Charu Thakur(171817) at Jaypee University of Information Technology, Waknaghat, India, is a bonafide record of theirwork carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.



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DECLARATION

We hereby declare that the work reported in the B. Tech. thesis entitled **"Green synthesis and Characterization of Phyto -Metallic Quantum Dots""** submitted at Jaypee University of Information Technology, Waknaghat, India, is an authentic record of our work carried out under the supervision of Dr. Garlapati Vijay Kumar, Dept. of Biotechnology and Bioinformatics, JUIT, Waknaghat, HP-173234, India. We have not submitted this work elsewhere for any other degree or diploma.

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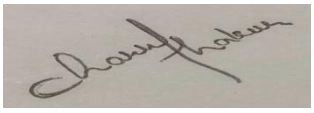

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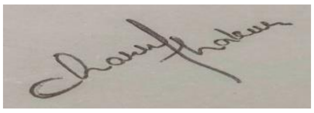

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ABSTRACT

The present work summarizes the state-of-the-art in synthesizing the Phyto-based metallic quantum dots. We have taken copper as a metallic compound and Artemisia nilagirica as a phytochemical source as a prototype and summarized the simple production approach and characterization of copper quantum dots from leaf extracts of the plant. Green fabrication of quantum dots includes green chemistry, which provides mild reaction conditions that help reduce the metal copper into a nanoparticle of a specific size compulsorily equal or below 10 nanometres. To confirm the production of copper Quantum dots, the product will be made to undergo several processes such as FTIR, SEM, XPS, TEM, SAED, etc. These will help determine the size and the surface chemistry of the quantum dots that help determine their efficiency and precision. The plant extract of Artemisia nilagirica would provide phytochemicals that contribute to essential reduction, stabilization, and capping agents of the copper Quantum dots. Further, the efficacy of synthesized metallic quantum dots was drawn through the catalytic, antimicrobial, and antioxidant activities. The catalytic activity was measured with the methylene blue dye-based test. The antimicrobial activity would be tested against Escherichia coli and Staphylococcus aureus. The antioxidant activity of the synthesized metallic quantum dots was determined through the DPPH test. The following study shall provide a state-of-art approach towards the synthesis and characterization of metallic quantum dots for a sustainable world.

Key words: copper, Quantum dots, Artemisia nilagirica, , antimicrobial, antioxidant, green synthesis.

1.INTRODUCTION

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One of the major aspects of science and medicine has always been diagnostics, imaging and therapy. These are the basic necessities for research and development in any field. From visualising samples under microscope to tracking genes with the help of markers, science has truly evolved over the years. With introduction of new techniques also arise new problems. We always have to strive to improve all aspects of any new technique to make it as safe as possible.(Kubra, Salman et al. 2021) It has to be ensured that it does not pose any harm to the environment or to the subject of the procedure. One of the most widely used compound in imaging, diagnostics and prognostics etc is a fluorophore.

Fluorophore is a chemical compound that, on excitation, can re emit light. Fluorophores can be used as a probe, a dye, as a tracer etc. They are mostly used for staining tissues or cells in methods like fluorescent imaging or spectroscopy. These compounds and other organic dyes have been in use for a very long time but they have certain limitations which may hamper the results.

Some of the limitations are as follows:

- The general organic dyes show multiple peaks also called ‘shoulders' which do not give accurate results or readings. Organic dyes do not have broad absorption spectrum. Organic dyes do not have high Quantum yield and are also extremely sensitive to light.(Wang, Bao et al. 2020)
- Organic dyes undergo fast photobleaching hence the imaging has to be carried out immediately as these cannot be exposed to light for extended periods of time.(Yan, Wang et al. 2020)
- The fluorescence lifetime of these compounds is very low and they are not specific enough.(Zhou, Li et al. 2020)
- Use of dyes over a long period of time may cause toxicity in the subject.(Gidwani, Sahu et al. 2021)
- These compounds are also not easily degraded and may even be biohazardous.

2.FUNDAMENTALS

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2.1 What are Quantum Dots?

Quantum dots were discovered in 1980 by AlexicEkimov and Louis E. Brus. It is one of nanotechnology's most important discoveries. Quantum dots are semiconducting nanoparticles that can pass electrons. They emit light if different colours depending on the structure and size of the particle. The Quantum dot can be tuned to emit light of a specific colour. They can be prepared used a variety of elements such as silver, gold, copper etc. (Mariselvam, Ranjitsingh et al. 2014)

They are an excellent replacement of the organic dyes and fluorophores used in imaging. Quantum dots can be used as probes, markers, tracers, tags etc. They help to combat many obstacles due to nature of organic dyes such as, they are more photo stable, they have a broad absorption pattern, resistant to photo bleaching, have a long fluorescence life, has specific binding and shows specific peaks and no shoulder effect. Quantum dots produced by green synthesis or layered by ZnS have shown very less toxicity when compared to organic dyes which were used over long time periods. They display fluorescent properties because of quantum restriction. The Quantum captivation occurs when a substance is diminished to a dimension like the characteristic linear measure of the attribute being analyzed. For Quantum Dots, the aforementioned trademark linear measure is: exciton Bohr radius, accordingly QDs on a similar dimension spectrum as their substance'strademark exciton Bohr range show quantum captivation.Quantum Dot fluorescence starts from the rejoining of an e-opening set, and the fluorescence is started via photo-excitation of an e- from the quantum dot valence band within its conduction band.The e- unwinds towards themost reduced energy state in the conduction band prior to amalgamate with the opening abandoned wherein the valence band. The distinction that is there in the energy allying the conduction and valence band stayspreserved as a discharged photon, subsequently, the size of this hole directs frequency of the light produced out of the quantum dots. The QD bandgap is straightforwardly identified with its diameter, and thusly QDs including a similar semiconductor substance can be attuned to radiate various frequencies by essentially converting their size.

Optical portrayal of the quantum dots is generally shown by UV-VIS and PL (i.e., photoluminescence spectroscopy), which provide quick, not destructive as well as contactless option. The photo modulated reflectance spectroscopy is an uncommon method utilized by Hazdra and associates. for checking QD structures that are deposited. This method gives equal energy resolution as far as the photoluminescence at reducedfebricity and experiment an additional extensive scope of critical focal points, featuring the low energy state or ground state and furthermore numerous high request inter-band optical advances through which the band structure, especially of the dampcoating, can be well thought out. The ocular characteristics (or the incandescent emanation) of quantum dots can be adapted by the quantum dots' dimension i.e. is a key specification that will decides the spectral location as well as the immaculateness of PL. Quantum Dots' dimension is commonly determined utilizing traditional procedures like SEM technique - scanning electron microscopy, TEM-transmission electron microscopy, and DLS studies- dynamic light scattering. In order to screen the dimension of the epitaxially arranged QDs, a few portrayal strategies are generally utilized, for instance transmission e- microscopy, AFM: atomic force microscopy / STM.

Moreover, quantum dots bio-compatible nature is fundamental to their natural and biosciencesignificance. All in all, bio-compatible qds can therefore be acquired through 3 unique highways:

- Bio-mimetic synthesis: either via the utilization of artificial cellular construction/bio-molecules for example nucleic acids, peptides, proteins, and catalystsas layouts;
- Bio-synthesis: utilizing alive life forms in bio-reactors;
- Changing the outside of quantum dots from their synthetic merger. The bio-synthetic methodology gives a green route to getting ready bio-compatible quantum dotsfree from producing harmful items or alternativelyhostile response state, while the exterior alteration proposition can construct an inflated QY to an excessive extent.

2.2 Classification of Quantum Dots

Table 1. Classification of QD

QD	Group no	Examples
Single core structure	II-VI	CdS, CdSe, ZnSe & CdTe
	III-V	GaN, InP & InAs
	IV-VI	PbSe & PbS
	IV	Ge & Si
Core/Shell structure	Type 1	CdSe/ZnTe, CdTe/ CdSe, ZnSe/CdS
	Type 2	CdSe/ZnS, InP/ZnS, CdSe/CdS/ZnS, CdSe/CdS/Cd0.5Zn0.5S/ZnS
Alloy		CuXZn1-XS, HgXCd1-XTe, ZnXCd1-XSe

Table 2.1: classification of QDs on the basis of elements used.

2.3 Applications

- They are used in LEDs, solar cells, lasers etc.(Molaei 2020)
- They are also used albeit particular markers for cellular construction and particles, discovering cell parentage, keeping a track of physiological incidents in animate cells, calculating cell movability as well as trailing cells in vivo.(Pandey, Bodas et al. 2020)
- If we compare QDs with organic dyes, they are photostable, with longer fluorescence life, broad absorption patterns, and give clear peaks for accurate visualisation.
- By tuning the structure we can make it emit light of the colour of our choice.
- Bioimaging
- Photovoltaic devices
- Light emitting devices

2.4 QD VS NANO

Depiction of nanoparticles is routinely associated to the localizing function of light in nanosized minuscules or networkswhichusually showcase a colossal field magnification at their exteriors.Common models are minuscule sole photonic segments, for instance, intricate nano radar, nanogaps, and also nanoresonators. Compact confinement is thus of phenomenal reasonable significance, for instance for elevated resolutionsurvey,regional alteration of substances, big field localization, strength elevation, increment of productivity of computational cycles, for example, Raman scattering and harmonic generation.

Nano-specks of semiconductor substancesput together in bandgaps are referred as quantum dots. Quantum dots (QD) are the nanocrystalsthat are made out ofthe semiconductor substances which are adequately little to display quantum mechanical characteristics. Quantum dots are employed in applications such as photovoltaic cells, light emitting diodes, and distinction specialists in diagnostics.(Nirala and Shtenberg 2020)

2.5 Elements used for the preparation of Quantum Dots

Some of the examples of the elements used for the preparation of QDs are: cadmium selenide, cadmium telluride, zinc cadmium selenide, zinc sulfide, and lead selenide, tobetter their optical characteristic, moreover a cap to permitupgraded aqueous dissolvability for bio associated applications.

2.6 QD'S as Biomarkers

Biomarker probes might be supportive for the filtering and detection of malignancy if a set of molecular probes can be measured and scientifically alteredin the middle ofoncogenic and healthy cells. Probes of sickness are frequentlyexisting at very plungingassemblies, so techniquesefficient enough for low detection limitation are needed. QDsare emanate autofluorescence on excitation with a light source. They consist of exceptional optical characteristics including high brightness, obstruction to photo-bleaching process and tunable frequency. Now a days advancement inexterioralteration of quantum dots allow them to potentially be utilizedin cancer diagnostics. Quantum Dots having near-infrared emission could be utilized for the process of sentinel lymph-node mapping to assistbiopsied and operations. (Zhang, Ba et al. 2020)Union of qds with bio-particles, plus antibodies as well as peptides, could be utilized for targeting tumors *in vivo*.(Freitas, Neves et al. 2020)A great amount of research is going on in the advancement ofQDs for cancer diagnostics and therapy from a scientific point of view and the for further betteringof QDmechanisms to visualize and target metastatic malignant cell growth, and to be able to quantify the measure of explicittarget pointson malignant growth by delivering bio-dynamic probes for target hindrance.

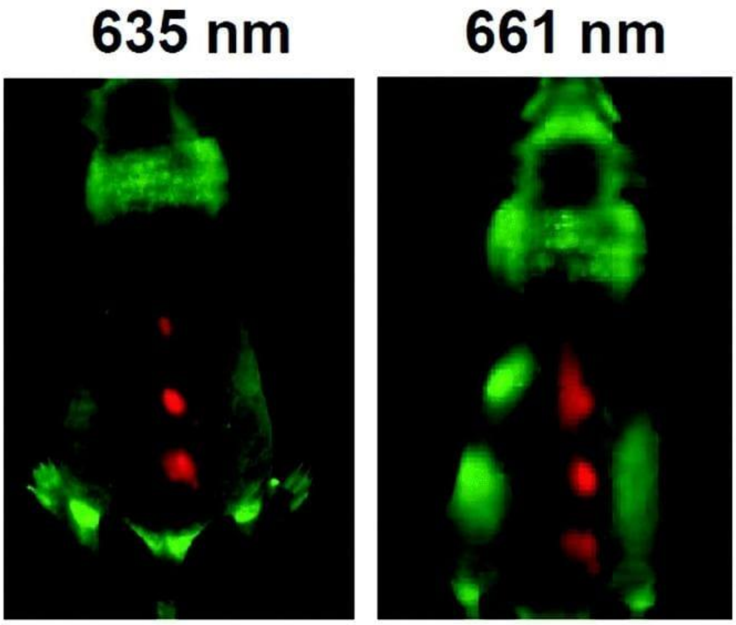

Fig 2.1: QDs as biomarkers

2.7 QD's instead of Natural Dyes

In contrast withnatural dyes, quantum dotsshowcase the alluringcharacteristic absorption property thatgradually rises towards smallerfrequencies. (beneath1stexcitonic absorption band) and a restricted emission band that is symmetrical in shape. The positions of absorption and emission spectraare attunable by particle dimension (i.e., quantum size effect.)Breadth of the emission spectra is specifically, for the most partregulated by the conveyance of the size of QD.The wide absorption permits unbound choicewhen it comes to the excitation frequency and in this mannerdirect division of excitation and emanation.(Al-Zahrani, El-Shishtawy et al. 2020)Themolar absorption (which is size dependent) quantities at the very 1stabsorption band of quantum dots are mostly hugewhen contrasted with natural dyes.(Jang, Kim et al. 2020)

Intriguingly, natural dyes also showcase high fluorescence quantum yields in the visible light reach however,in the best case scenario, average in the NIR frequency spectra. Now the combined downsides of decreased quantum yield at NIR frequencies as well as therestricted photostability of various NIR-frequency dyes interferes with the utilization of natural dyes for NIR-frequency fluorescence imaging. When contrasted withnatural dyes, another reliant, bi- or multiexponential QD decay behaviorrenders species ID from time settled fluorescence estimations extremely troublesome. This, unfortunately, is an innate disadvantage of these materials.

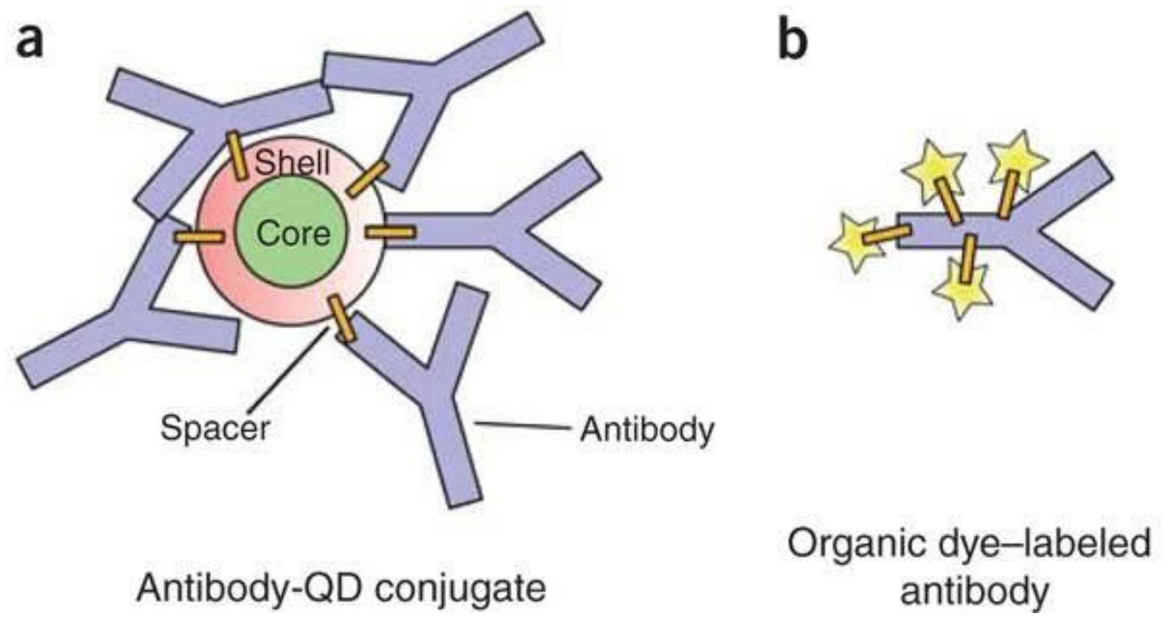

Fig 2.2 : QDs vs Organic dyes

2.8 Advantages of QD's over conventional Fluorophores

Quantum dots provide a few benefits over natural fluorophores which are ordinarily utilized for in vivo labelling. Quantum dots can have a wide scope of optical properties not present in natural fluorophores, for example, rhodamine and fluorescein.(Ahmed and Emam 2020) Natural/ Organic fluorophores have a restricted absorption range which brings about a tight scope of outflow. Additionally, the natural dyes don't have a sharp symmetric outflow top which is additionally expanded by a red-tail. In contra quantum dots offer a few benefits over natural fluorophores which are ordinarily utilized for in vivo naming. Quantum dots can have a wide scope of optical properties not present in natural fluorophores, for example, rhodamine 6g and fluorescein. Natural fluorophores have a tight absorption range which brings about a thin scope of discharge. Likewise, the natural dyes don't have a sharp symmetric emanation peak which is additionally expanded by a red-tail. Conversely, quantum dots have a more extensive excitation range and a tight more forcefully characterized outflow peak. Because of these properties, a solitary light source can be utilized to energize multicolor quantum dots all the while without signal cover st, quantum dots have a more extensive excitation range and a narrow all the more pointedly characterized emission top. Because of these properties, a solitary light source can be utilized to energize multicolor quantum dots all the while without any overlaps.(Li, Luo et al. 2020)

2.9 Characteristic properties of QD's

It is a semiconductor nanocrystal which is recognized for its bandgap energy, that is the energy needed to energize an e- from one electronic band to another at a relatively higher energy level. The excitationconspire eventually makes an e-opening pair also be called as an exciton. After unwinding back to its ground state, the exciton emanates energy as a fluorescent photon. At this point, the semiconducting nanocrystal measure gets like the mass of the Bohr exciton span, which is a trademark dimension lying in the scope of 2-10 nm, the molecule acquires unique electrical as well as optical characteristics.

- **Photostability:**Photostability of QDs generally relies upon surface passivation which is dictated by the QD covering atoms. Photostability of semiconductor QDs is allegedly higher than that of natural dyes, however QDs may likewise be influenced by the exposure of light. The result of such exposure may rely upon numerous trial factors, can prompt either an increment or decrease in the photoluminescent proficiency of QDs and is hard to anticipate.(Kulakovich, Gurinovich et al. 2020)

- **Absorption:**By and large, the optical retention peaks of QDs in the UV-region is typically assessed as π-π* change of sp2 formed carbon and n-π* change of hybridization with heteroatom like N, S, P, and so forth. The absorption characteristic can be controlled through surface passivation or alteration measure. Jiang et al. fostered a simple aqueous technique to integrate red, green and blue radiant CQDs by utilizing three isomers of phenylenediamines. The UV-visible absorption spectra of the as-acquired CQDs showed undifferentiated from design. Curiously, the absorption changes of these three CQDs were red-moved, showing the electronic bandgaps of the CQDs were more compact than their relating antecedents.(McBride, Mishra et al. 2020)

- **Photoluminescence:** Photoluminescence is perhaps the most captivating highlights of QDs, both from the perspective on central exploration and practical application. All in all, one uniform component of the PL for QDs is the particular reliance of the outflow frequency and intensity. The justification this exceptional marvel might be the optical determination of nanoparticles with various size or QDs with various emissive snares on the surface level .The variety of molecule size and PL outflow can be reflected from the expansive and excitation-subordinate PL emission range.(Nie, Jiang et al. 2020)

- **Electroluminescence:** Since semiconductor nanocrystals are notable to show electroluminescence (ECL), there ought to be nothing unexpected that QDs have enlivened different interests for ECL contemplates which can well be utilized in electrochemical. One example is, Zhang et al. (2013) revealed a CQDs-based light-emanating diodes (LED) gadget, in which the outflow tone can be constrained by the driving current. Shading switchable ECL from a similar CQDs going from blue to white was seen under various working voltages. (Shu, Lin et al. 2020)

To comprehend the iridescence component of CQDs all the more plainly, the scientists proposed two models dependent on the band gap emanation of the formed p domain and the edge impact brought about by another surface imperfection (defect). The PL qualities of the fluorescence emanation of QDs from the formed p space are gotten from the quantum confinement effect (QCE) of p-formed electrons in the sp2 nuclear structure and can be changed by their size, edge arrangement, and shape. Fluorescence discharge of CQDs related with surface deformities results from sp2 and sp3 hybridized carbon and other surface imperfections of QDs, and even fluorescence power and pinnacle position are identified with this deformity.

2.10 *Artemisia Nilagirica*

Artemisia nilagirica, is a well-known plant that is frequently referred as the Indian wormwood; belonging to the family - Asteraceae, it can be seen in the hilly regions of India. It is stretched outall throughout the Himalayan regionofIndia. With it is characteristic towering aromatic bush, it is used as amedicinal herbis. It hasleafy and branched stems. Leaves can be observed as: large,alternate,ovate and lobbed, greatly pinnatisect with small looking stipule-like lobes at the very base and with pubescent above, almost ash-grey or white-tomentose underneath; higher mostleaves are a bit smaller looking, 3-fid or entire, lanceolate. Flowers can be observed as small and they stand in extensively narrow clumps at the very top of the stem, sub globose heads, in spicate/ suberect/ parallelpanicled racemes. The color seen isbrownish yellow. Flowering tops as well as the leaves are: bitter, astringent and aromatic. Fruits, on the other hand, are minute like bracts ovate or oblong. The oil percentage and the yield of oil differs as the distribution of the plant, moreover, italso depends on the growth phases.(Ijaz, Zafar et al. 2020)

The plant shows admirably important pharmacological attributes. Traditionally, it is use is seen in the management of epilepsy; nervous disorders,as diuretic; anti-inflammatory aas well as skin disorders. Over the years, a large number of actions of aerial parts are examined and studied carefully and it was observed that they possess important properties like:anti-microbial, anti-fungal, antibacterial, ant filarial, in-secticidal, anti-ulcer, anti-malignant, anti-oxidant and anti-asthmatic properties. Some essential oil constituents in *A. nilagirica*. are Camphor, 1,8-cineole, β-eudesmol, artemisia alcohol, α-gurjunene, para-cymene, terpinene-4-ol and α-pinene. (Wang, Qi et al. 1993)The Oil possesses anti- microbial, anti-fungal, in-secticidal and larvicidal properties.All of these evidences just confirm that the whole plant is of great therapeutic value.(Odey, Iwara et al. 2012)

Fig 2.3: Artemisia nilagirica

2.11 Why only copper?

Copper is a low-cost metal that is readily available everywhere. Moreover, CuQDs can be efficiently exploited as catalysts, antioxidant and antimicrobial agents.

2.12 How it would be economical?

There’s a need for a new, innovative and efficient as well as cost effective method for the breakdown of natural dyes and to be able to keep a checkon pathogenic microbial growth in wastelands of various industries.

OBJECTIVES OF THE STUDY

1. Synthesis of copper quantum dots (Cu'QDs) of *Artemisia nilagirica.*
2. Characterization of Cu'QDs of *A. nilagirica*
3. Evaluation of catalytic, antibacterial and antioxidant activities of Cu'QDs of *A. nilagirica*

3. PREPARATION & CHRACTERIZATION OF QD'S

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Nanoparticles can be produced by various methodologies such as physical or mechanical, chemical or green synthesis. Other than green methods the other two generally include toxic pre cursors and may lead to production of by products or waste products that could be hazardous to environment. Sustainable building is of utmost importance and hence, this is where green synthesis comes into play.(Matussin, Harunsani et al. 2020)

Green synthesis is the utilization of environmentally congruent materials that can be microbes such as , fungus or plants for the synthesis of nanoparticles or in this case Quantum dots.(Chand, Cao et al. 2020) It eliminates the need of toxic precursors and uses sensitive and mild reaction conditions.(Olaokun, Alaba et al. 2020) It also minimizes waste production, is environment friendly and the whole process is clean and non toxic hence it is one of the most effectively sustainable methods of producing nanoparticles are Quantum dots.

3.1 Fabrication of Copper Qauntum Dot's

3.1.1 Preparation of plant extract: Leaf extracts can be prepared by various methods the most prevalent of which are hot water extraction and ethanol extraction.(Abubakar, Haque et al. 2020)

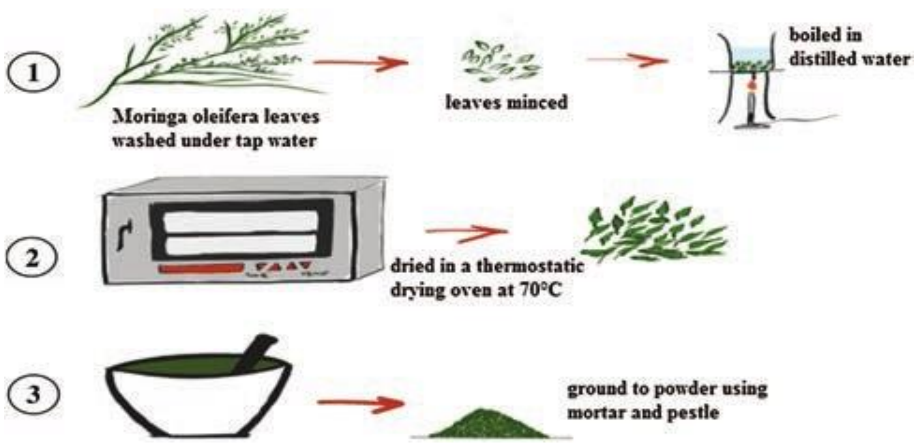

Fig 3.1 : Steps to make powder of the leaf to be used for extraction

3.1.2 Making of Hot water extract

To make plant extract by this process we need to first drive the leaves completely and then make fine powder out of it. 10 the leaf powder is wait and put in in hot water to soak and is boiled for 30 minutes and put aside to cool for 24 hours. (Chand, Cao et al. 2020)

Fig 3.2: Hot water extraction of plant component

3.1.3 Ethanol extract preparation

In ethanol extraction the Powder of the dried plant it is taken and so in in ethanol to completely submerge it. This mixture is left inside and ultra low temperature freezer for about 24 hours. In these 24 hours all the soluble components will be separated by the ethanol and thus this way the plant extract would be formed and can be further used for the preparation of quantum dots.(Andleeb, Alsalme et al. 2020)

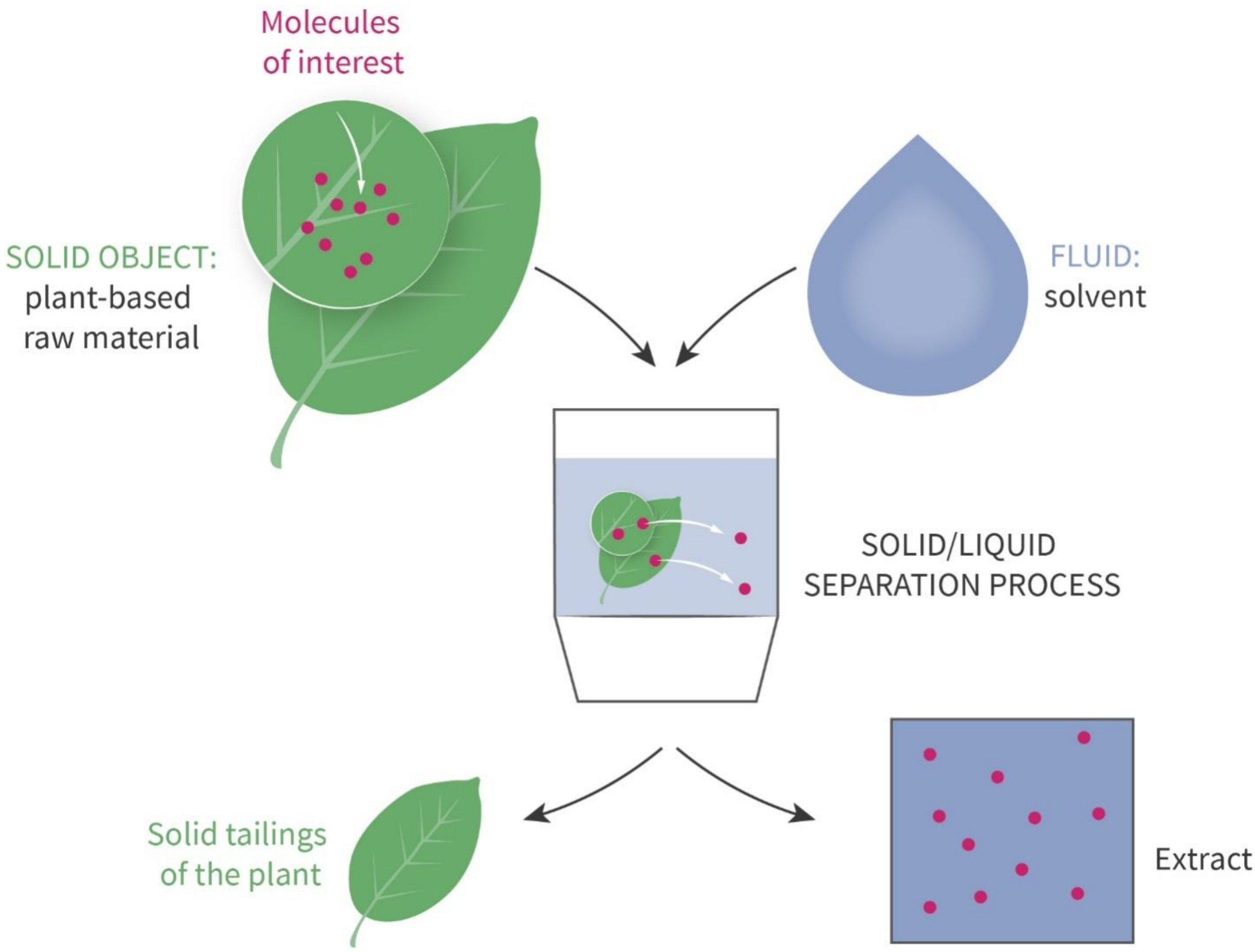

Fig 3.3: Ethanol extraction process.

3.1.4 Final Fabrication step of Quantum Dots

After the leaf extract has been successfully prepared it is now used for bio fabrication of copper Quantum dots. The first step in the preparation is to make a fresh batch of $CuSO_4$. (Romanik, Gilgenast et al. 2007). The filter plant extract it is then mixed with this copper sulphate solution with the help of magnetic stirring at room temperature. The reaction slowly turns to yellowish and then brown colour in about 10 minutes. This confirms successful fabrication of the copper Quantum dots.(Rani, Singh et al. 2020).

Fig 3.4: Workflow for green synthesis of nanoparticles using plant leaf extract

However, for the optimization of the experiment many parameters had to be observed at different values.

- Firstly, the quantity of the leaf extract to be used was determined by making batches from 1 ml to 4 ml separately and efficiency was observed.
- Next the effect of quantity of copper sulphate solution was observed. The concentration was varied from 1 to about 5 mM.

- The temperature ranges were also observed from 30 degree Celsius to 60 degree Celsius with equal intervals.
- The pH was also determined by using a wide range starting from 6 to 12. 0.01M of HCL and NaOH were used to regulate it.
- The optimal incubation time was also determined by observing the experiment at different time intervals starting from 10 minutes to 96 hours.

3.2 Determination of therapeutic and catalytic efficacy of QD's

3.2.1 Determination of antibacterial activity

Firstly, the antibacterial evaluation of Cu QDs was done and it wasthen analyzed against the strains of E. coli KT45/45A and *S. aureus* KT68,respectively. Shortly, followed by the technique of agar well diffusion. Agar slants of supplement media were formed and then were utilized for keeping up the cultures of *E. coli* and *S. aureus* prepared in stock at about 4 C. Prior to tests, the unadulterated pure cultures were then sub-refined onto nutrient agar inclinations which were afterwards put into incubation for the time being at 37 C. (Jose, Anilkumar et al. 2020)Afterward, the nutrient agar plates were arranged and then inoculation was done with one or the other strains of *E. coli/ S. aureus* and then followed by incubating for 24 h. It brought about even development (as seen all over the supplement nutrient agar plates). They were then punctured for wells and enhanced with - 50 µL Cu QDs. The growth inhibition zones could be seen after 24 h of incubation period. Additionally, the controlled activities of *M. indica* leaf extricate just as a mass of Cu2SO4 composite was examined.(Alavi, Jabari et al. 2021)

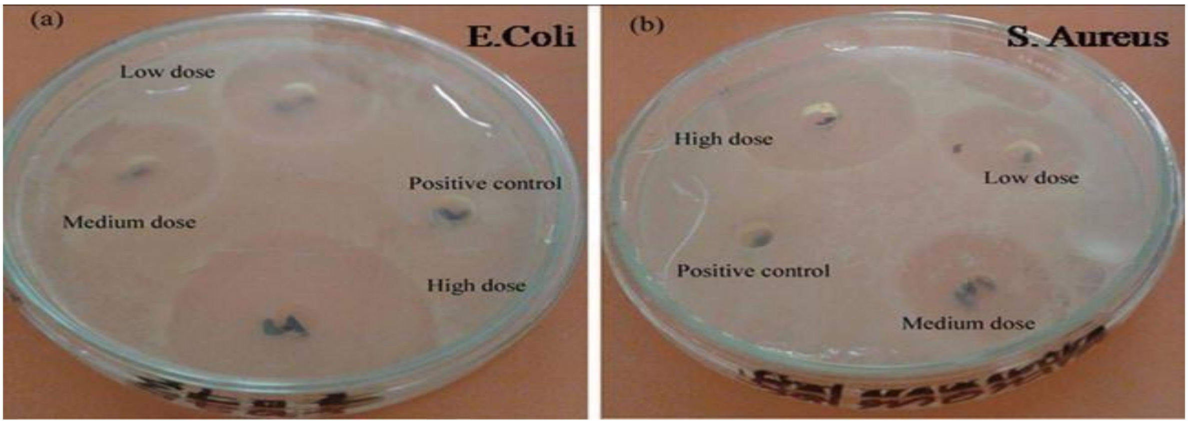

Fig 3.5: Antibacterial activity of CuQDs

3.2.2 Determination of antioxidant activity

- **Approach 1:**

  The antioxidant activity of Cu quantum dots was assessed with little adjustment utilizing DPPH: 1,1-diphenyl-2-picrylhydrazylwas used as a free radicle model.DPPH provides a method that is speedy forevaluating theantioxidant property. Here, in the examination, different makeups - (250, 500, 750 and 1000 μL) of M. indica leaf extricate or/ Cu QDs/M. indica leaf extricate reaction blend were added to a 2000 μL of 0.1 mM DPPH arrangement in the presence of pure methanol with option of dst.H2O in order makeup the last volume upto3000 μL. Resultant reaction blend was then shaken energetically, trailed by incubatingfor 10 min at 37 C in the dark. Control was already set up by adding 1000 μL of distilled H2O to 2000 μL DPPH arrangement.(Jovanović, Dorontić et al. 2020) The color change can be observed as follows: violet → yellow; it was a recognized attributable to its anti-oxidation potential. Moreover, the optical thickness of the reaction blend was resolved at 517 nm. The formula used for determining the free radical scavenging activity was:

  Scavenging activity→ (%) = ¼ {[1- Absorbance of sample/Absorbance of control] x 100}

- **Approach 2:**

  The antioxidant properties were researched utilizing two unique kinds of free radicals, to be specific, superoxide and hydroxyl. Superoxide radical elimination productivity was resolved utilizing the autoxidation of pyrogallol technique, while OH- ones were controlled byFenton reaction. For determining the capacity of free radical scavenging (denoted as S), the conditions that were utilized are as follows:

  $$S_S \rightarrow \% = \{[(A_S - A_0)/A_0] \times 100\%\}$$

  Where:

  S_S: scavenging activity to remove superoxide radicals (%)

  A_0: - standard absorbance, (-)

  A_S: - sample absorbance, (-)

  $$S_{OH} \rightarrow (\%) = \{[(A_S - A_0)/ (A - A_0)] \times 100\%\}$$

  Where:

  S_{OH}: - Scavenging activity to remove OH- radicals, (%)

  A_0: - Std absorbance

  A_S: - Sample absorbance

  A: - Solution without $FeSO_4$ absorbance

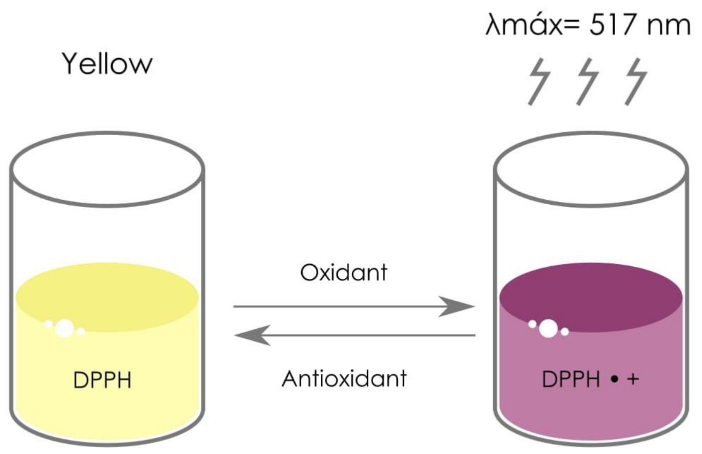

Fig 3.6 : Antioxidant activity of CuQDs

3.2.3 Catalytic activity determination

Copper Quantum dots would be considered good catalysts if they significantly improve the rate of reaction of any experiment there used in. To test this we perform an experiment that observes catalytic activity of the above found Quantum dots by the extent of their reduction capacity for MB. Three experiments were designed and their absorbents or optical density was measured and inference was made. (Aqeel, Ikram et al. 2020)

For determining the catalyst activity of the QDs, three experiments were set up.

- In the first setup MB along with distilled water was taken and its absorbance was measured.
- In the second set up MB and distilled water supplemented with leaf extract of Artemisia nilagirica. Absorbance was noted.
- In the third setup MB along with distilled water was supplemented with copper Quantum dots and the absorbance was measured.
- The optical densities obtained by the first two experiments were compared to the third one.

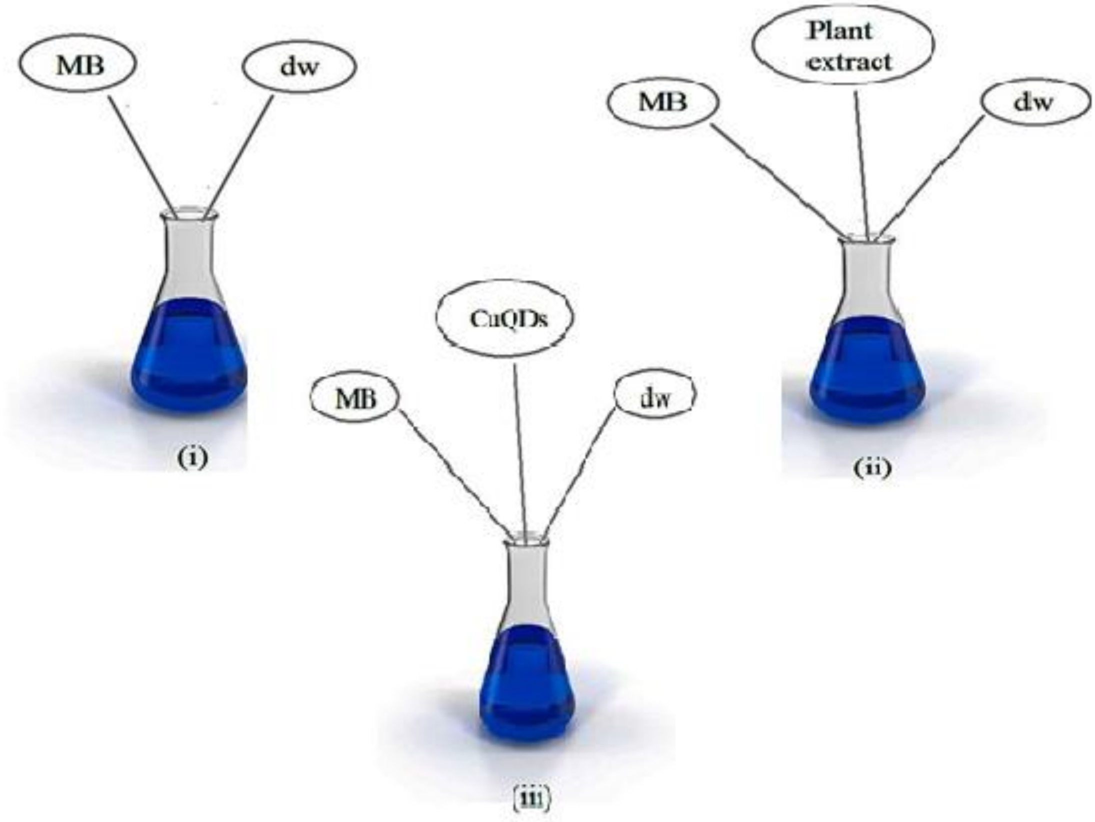

Fig 3.7: Setup"S in QD's catalytic activity determination

3.3 Characterization Techniques for QD's

Definite nuclear scale portrayal of QDs is subsequently important to improve their optoelectronic properties. Distinctive portrayal procedures that can be utilized for measure of the dimension, shape and anatomy of the QDs are depicted in this part. Every portrayal procedure has its own benefits and constraints in giving primary and creation data of the QDs.

3.3.1 Atomic Force Microscopy

It comprises of a cantilever tip connected to a piezo-actuator to examine across the surface. The nuclear powers between the tip and the surface causes a quantifiable avoidance of the cantilever which is utilized to repeat the surface geology. By and large an optical switch strategy is utilized to identify the cantilever redirections, where a laser shaft is centered around the rear of the cantilever and the reflected bar is gathered by a photodiode. The laser bar diversion framework is associated with a criticism circle to control the power and the situation of the tip. AFM can be worked in 3 unique modes: (i) Contact mode: - where the head is consistently touching the surface; (ii) Non-contact mode: - where the head wavers over the surface and also the forces were estimated; (iii) tapping mode: - where the head contacts the surface intermittently swaying near the reverberation recurrence. At brief good ways from surface the van der Waals forces are available which draw in the tip toward the surface. At the point when the distance of the tip from the surface is additionally diminished, the horrendous forces because of the collaboration between electronic clouds of tip and the surface become prevailing.(Khan, Nayan et al. 2020)

3.3.2 Transmission Electron Microscopy

TEis a high goal imaging strategy in which an electron beam is communicated through a meager sample. The association of transmitted electron beam with the sample delivers the picture. The electron source and the identifier are situated on inverse sides as for the example. The e-beam communicates through the sample and afterward is caught and prepared by the detector(s). The connection volume of the e-beam is little and comparable to the de Broglie frequency of the electrons. On a fundamental level, TEM can distinguish the situation of cross section planes in a crystalline solid and can deliver the lattice diffraction design also. The QDs can be recognized by the dark pyramid like highlights in the splendid network. In a unique report electron microscopy has been utilized to make a 3D tomographic picture of a QD. (Marin, Skripka et al. 2020)

In any case, test readiness and acknowledgment of appropriate diminishing for TEM examination is profoundly mind boggling and tedious. During the readiness of a meager lamellae of the sample by utilizing miniature and nano-machining procedures or utilizing focused ion beam (FIB), there is a high likelihood to change or harm the construction of the example. The local data is restricted and the acquired outcomes are found the middle value of over the entire lamellae where likewise nearby strain fields can influence the picture contrast. Extra strategies are expected to determine the local changes in the morphology and organization at the atomic scale.

3.3.3 Cross-Sectional STM: Scanning Tunneling Microscopy

STM was formed by Binnig and Rohrer in the year 1981 is an imaging strategy that can accomplish nuclear goal. It chips away at the standard of quantum mechanical tunneling through a vacuum obstruction. STM comprises of a sharp metallic tip looking over the surface with the assistance of piezoelectric stacks.(Zhang, Jin et al. 2020) A bias voltage needs to be applied in between the sample and the head(tip) and when the tip is sufficiently close to the surface, a quantifiable tunneling current is recognized. At negative sample bias, the electrons tunnel from filled conditions of the sample to the tip (i.e., filled-state imaging) and at positive bias voltages the electrons tunnel from tip to the vacant conditions of the example (or void state imaging). The tunneling current is amazingly delicate and dramatically rots with the tip-test distances. STM can be worked in 2 unique ways: (I) Constant Current (I)mode: - where the tunneling current, is kept consistent and the distinctions in stature are estimated; (ii) Constant height mode: - the stature of the head is kept steady and estimating the tunneling I. An input circle is then utilized to keep up either steady height or consistent current and to change the tip-test distance to keep up either consistent current or consistent stature.(Castro, Ribeiro et al. 2020)

4. Conclusions

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- The present state-of-art provides the basic, conservative, fast and green engineered convention for the high bio-prompted change of Cu2þ particles into tiny measured and round formed Cu QDs alongside Cu2O as minority stage utilizing *Artemisia nilagirica*.leaf separate alongside which the cycle can be scaled-up.
- The propsed fabrication approach, there isn't any surfactant, covering specialist and format that were utilized.
- The Polyphenols (basically mangiferin) of the plant *Artemisia nilagirica*.leaf remove was principally which was responsible for the arrangement of copperquantum dotswhich went about as lessening/covering specialists.
- The investigation exhibited, the assynthesized copper quantum dots can be potentially misused like an effective antioxidant and antimicrobial and catalytical specialists.
- The copper quantum dots so-delivered were not only just very viable as the antimicrobial specialist against the *E. coli/S. aureus* strains yet additionally portrayed the fast decrease of Methylene Blue, recommending that copper quantum dots may be productively utilized in industrial wastewaters both as antimicrobial agents for the removal of the growth of the pathogenic microorganisms and impetus for the expulsion of harmful, non-biodegradable, anthropogenic toxin methylene blue and some other natural dyes, consequently providing the chance for the treatment of wastewater in relationship with other progressed methods for applications such as horticultural yields water system, etc.
- In view of the current investigation, these QDs could be additionally utilized as particular cancer prevention agent, Cu-based dressing or /antimicrobial coatings for injuries what's more, elective material against various medications safe pathogenic microorganisms, bioterrorism-connected contaminations, and so on.
- Be that as it may, further investigations are needed to totally describe the properties identified with sensor alongside cytotoxicity and instrument of activity connected to the cancer prevention agent and anti-bacterial action of copper quantum dots.
- These examinations will not only be helpful in advancing sensors based on copper QDs, yet additionally for surface change, in this manner encouraging medication stacking on the copper quantum dots (with extra focal points of anti-microbial and cell reinforcement attributes).(Rani, Singh et al. 2020)

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