Green synthesis of copper nanoparticles by *Cocos nucifera* (L.) shell extract

Project report submitted in partial fulfillment of the requirement for the degree of Bachelor of Technology

IN

BIOTECHNOLOGY

BY

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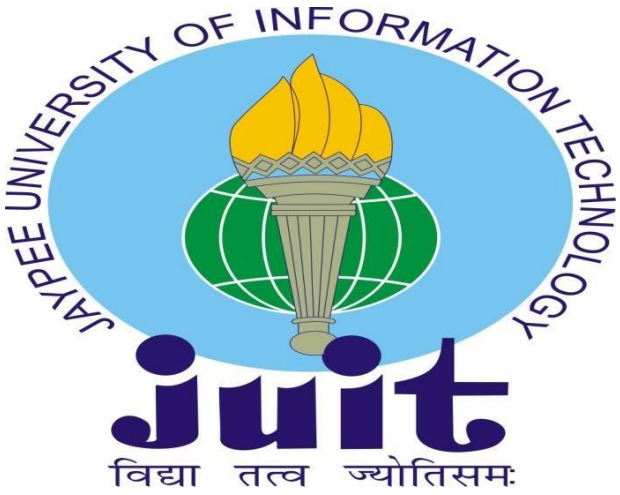
(201803)

Under the supervision of

Dr. Abhishek Chaudhary



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SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the project work entitle "Green synthesis of copper nanoparticles by *Cocos nucifera* (L.) shell extract" submitted by Vaibhav Tiwari (201803) at Jaypee University of Information Technology, Waknaghat, Solan, Himachal Pradesh, India, is bonafide record of his original work has not been submitted elsewhere for any other degree or diploma.

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DECALARTION

I hereby declare that the work presented in this report entitled "Green synthesis of copper nanoparticles by *Cocos nucifera* (L.) shell extract" in partial fulfilment of the requirements for the award of the degree of Bachelors of Technology in Biotechnology submitted in the Dept. of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat is an authentic record of my own work carried out over a period from July 2023 to May 2024 under the supervision of Dr. Abhishek Chaudhary, Assistant Professor (SG), Department of Biotechnology and Bioinformatics, JUIT.

I also authenticate that I have carried out the above-mentioned project work under the proficiency stream of Medical Biotechnology. The matter embodied in the report has not been submitted for the award of any other degree or diploma.

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ACKNOWLEDGMENT

I would like to express my heartfelt gratitude to the institution of Jaypee University of Information Technology for letting me explore my area of research and have hands- on experience in the world of science and technology. I would like to express my deep and sincere thanks to my guide coordinator Dr. Abhishek Chaudhary who helped me throughout my research constantly. His dynamism, vision, sincerity and motivation have inspired me a lot.

Secondly, I would like to thank the lab attendants who helped me whenever I got stuck in between tasks. Also, I am extremely grateful to all the teachers for their Support and enlightening me in the right direction.

Last but not the least; I am grateful to my co mates for providing me with a helping hand whenever it was necessary.

(Vaibhav Tiwari, 201803)

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LIST OF ABBREVIATIONS

Symbol	**Abbreviation**
℃	Degree Celsius
%	Percentage
mg	miligram
g	gram
NP	Nanoparticles
CuNP	Copper Nanoparticles
ml	Milliliter
µl	Microliter
dH2O	Distilled Water
$NaBH_4$	Sodium borohydride
SEM	Scanning Electron Microscopy
XDR	X-RAY Diffraction
PVP	Polyvinylpyrrolidone
PVA	Polyvinlyalcohol
TEM	Transmission electron microscopy
LSPRs	Localized surface plasmon
Cryo-TEM	Electron cryogenic transmission electron microscopy
MHA	Mueller Hinton Agar

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ABSTRACT

A multidisciplinary field called nanotechnology includes a wide spectrum of innovations that come from multiple fields. Fast development in science and technological progress have opened up the emerging subject of nanotechnology, which has numerous new prospects for improving medical research and illness treatment in the field of human health care. Further in this report, there is discussion about the synthesis of nanoparticles by chemical method and green method as it involves less usage of harmful chemicals. Implementation of nanoparticles are also discussed and some of future applications. Further discussion on green synthesis of copper nanoparticles by extract of *Cocos nucifera* (L.), potential use of its extract, its phytochemical composition and potential usage of *Cocos nucifera* (L.) in medical field. Greener approach is less expensive and it require less amount of resources and it also require less amount of time for the synthesis of nanoparticles.

CHAPTER 1

INTRODUCTION

NANOBIOTECHNOLOGY - The science and engineering processes engaged in designing, synthesizing, characterization and application of materials and devices whose functional structure is at least at one dimension at nanoscale. At this level the activities at nanoscale becomes important as well as interaction of nanoparticles. When we consider its applications in medical field , whole scenario shifts when we mix nanotechnology with biomedical field , then different implementations can be in the form of antibacterial and antifungal creams , wound healing , antioxidant , cancer treatment and drug target delivery , further through this technology, atomic or molecular grade machines can be created by imitating or incorporating biological systems, or by creating microscopic tools to research or modify certain molecular characteristics of a biological system. By fusing cutting-edge information technology & nanotechnology applications with current biological problems, nanobiotechnology may, thus, simplify several paths in the life sciences (Hudak et al.2012)The classification of nanoparticles can also be done by classifying them into carbon based nanoparticles , metal nanoparticles , ceramic nanoparticles, Polymeric nanoparticles and lipid based nanoparticles Various techniques can used to characterize nanoparticles like UV-spectroscopy ,XDR ,XPS, NMR, SEM, TEM, FTIR, Liquid TEM, Cryo TEM and TSEM moreover it can also be done on the basis of shape, size, optical properties, chemical structure, crystal structure and concentration . Our existing concepts and understanding could be altered by this technology, which has the ability to partially dissolve the barriers between biology, physics, and chemistry. Some of the application that can come handy: -

Diagnostic applications: The majority of diseases now diagnosed rely on the patient exhibiting observable symptoms before health care providers can determine that the patient has a particular ailment. However, the likelihood of a successful response to treatment may have decreased by the time those symptoms have shown. Therefore, the likelihood of finding a cure increases with earlier disease detection. In a perfect world, diseases would be identified and treated before any symptoms appeared (Majernik et al.2012).

Specific target probes: The medical community will continue to favor optical and colorimetric detection not with standing the benefits of magnetic detection. One of the businesses that created methods that enable physicians, detect the genetic foundation of biological material is Nanosphere (Majernik et al.2012).

Mainly there are three methods of synthesis i.e. chemical, physical and biological, mainly I have focused on aspects of biological synthesis.

In chemical synthesis: -

It mainly contains three major components- metal salts + capping agents + reducing agents. Here capping agent acts as stabilizers as nanoparticles are highly unstable, simultaneously also prevent agglutination nanoparticles and reducing agents acts as reduces the metal ions into nanoparticles. Heat may be provided if necessary. Examples of reducing - Sodium citrate, $NaBH_4$, Ascorbic acid. Examples of Capping agents can be Thiols, Citrate and Polymers: - PVP and PVA. There are two approaches of synthesizing nanoparticles are top down and bottom up.

In physical synthesis: - Laser ablation, sputtering, lithographic and mechanical methods can be used in order to synthesis nanoparticles from it.

In biological synthesis: -

Instead of using chemical reducing agents, environment friendly agents are used like starch as it can be used both as reducing agents and capping agents. The main advantage of using such types of methods that contamination free nanoparticles could be obtained and also can be used in drug delivery, skin care and cosmetic industry. Also, it can be synthesized in minimal resources which ultimately reduces the cost of production while making them.

CHAPTER 2

REVIEW OF LITERATURE

Synthesis of nanoparticles there are two approach of synthesizing nanoparticles that is top-down and bottom-up. In Top - down synthesis involves the converting large bulky particles into nano particles. For bottom - up approach it's just opposite (from small particles to nanosized particle synthesis). During this, two main stages involves i.e. nucleation and growth, in nucleation there is initial formation of first nanoparticles i.e. in the form of crystals and in growth it involves appearance of very small particles ,there may be two nuclei can be as observed homogeneous and heterogeneous nuclei, basic difference between these two is uniformity throughout solution and nucleation time, also in homogeneous particles mono-dispersed particles formation takes place ,on the other hand heterogeneous particles polydisperse particles will form. Mono-dispersed polymers are macromolecules materials having a precise and discrete molecular weight while poly-dispersed polymers are macromolecular materials having a range of components having a range of components with a range of molecular weight. Highly mono dispersed nanoparticles are formed in the process of nucleation and growth can successfully separated (Gopinath et.al 2017). American Physicist and Nobel prize winner first time introduced the concept of nanotechnology in 1995. He also delivered a lecture entitled "There's Plenty of Room at the Bottom".

If we talk about the aspects of nanoparticles growth in solution arrested precipitation is also a concerning matter. Ostwald ripening is a primary source of functional nanomaterial deactivation. It entails the formation of larger nanoparticles at the disadvantage of smaller ones. Nucleation-like events are required for the continuing stability of a collection of confined nanoparticle.

Another important aspect is tuning of the size of nanoparticles i.e. control over the nucleation and growth rate; faster will be the nucleation, higher will be the concentration of nuclei and lower will be the yield. On the counterpart slow nucleation may leads to lower concentration of nuclei and higher will be the yielding particles(Gopinath et.al 2017).

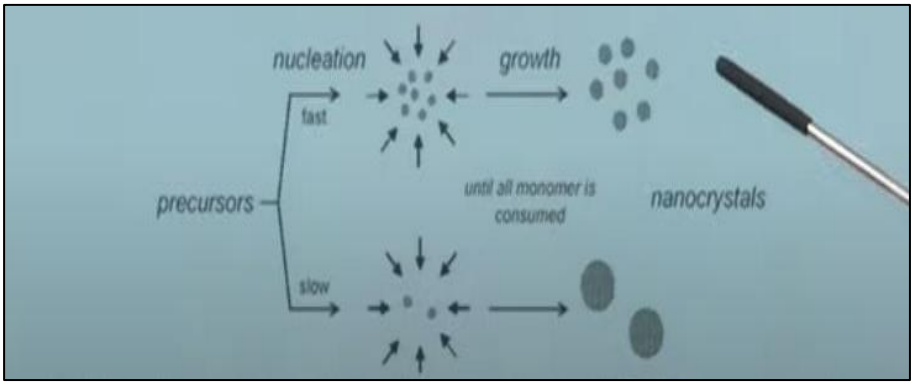


Fig 1 Flow chart type representation of nucleation process [1]

Tuning of nanoparticles can be categorized in two types :-

Anisotropic growth - Anisotropic nanomaterials are a type of material whose properties are direction-dependent and require more than one structural parameter to describe. Their distinct and decently physical and chemical features make them perfect candidates for developing novel applications.

Isotropic growth - In this class, the nanoparticles are independent of direction; shape and size of such particles are same and uniformity can be seen among all the particles.

Iodide Ion act as key to shape-directing elements because it can strongly and selectively bind to nanoparticles and favors anisotropic structures, starting with general component CTABr and iodide concentration deliberately adjusted to achieve target morphology. Seed particles can be used in the formation of all three nanoparticles architecture and final shape can be adjusted by controlling iodide concentration as concentration of iodide becomes (>10 µl) a mixture of different morphological nanoparticles can be seen containing (triangle , prism , rod and so on) (Millstone and Wei et.al 2014)

Roles of stabilizing agents: -

- Prevents uncontrollable growth of particles
- Prevent particles aggregation
- Control growth rate
- Control particles size
- Allows particles solubility in various

Parameters that effects particle growth:-

- Reducing agents
- Concentration of reactant
- Ph value of solution
- Duration of heat treatment

GREEN SYNTHESIS OF NANOPARTICLES

The importance of "green" chemistry and chemical processes has grown during the past ten years. Through the adoption of 12 guiding principles, these initiatives seek to completely eliminate or at the very least significantly reduce the amount of trash that is produced. Any effort to achieve these objectives must fully take into account these principles while designing a synthetic pathway, a chemical analysis, or a chemical process. A green synthetic strategy should give careful consideration to the use of nontoxic chemicals, environmentally friendly solvents, and renewable resources, among other essential factors. In the current work, we describe a completely eco-friendly method for creating metal nanoparticles (Raveendran and Fu et.al 2002). The three major phases in creating nanoparticles are assessed with spectrum of green chemistry: selection of solvent media utilized for synthesis, selection of an ecologically friendly reducing agent, Non-toxic substance used to stabilize the NPs (Raveendran and Fu et.al 2002). Algae, plant extracts, fungi, yeasts and bacteria is generally used for of nanoparticles and generally it is favorable to use plant extract as it requires less laboratory work. If algae, fungi, yeast and bacteria is used for the synthesis of nanoparticles then additional work has to be done like preparation of media for the growth of bacteria, fungi, yeast and bacteria, contamination free environment is necessary and storage has to be done properly.

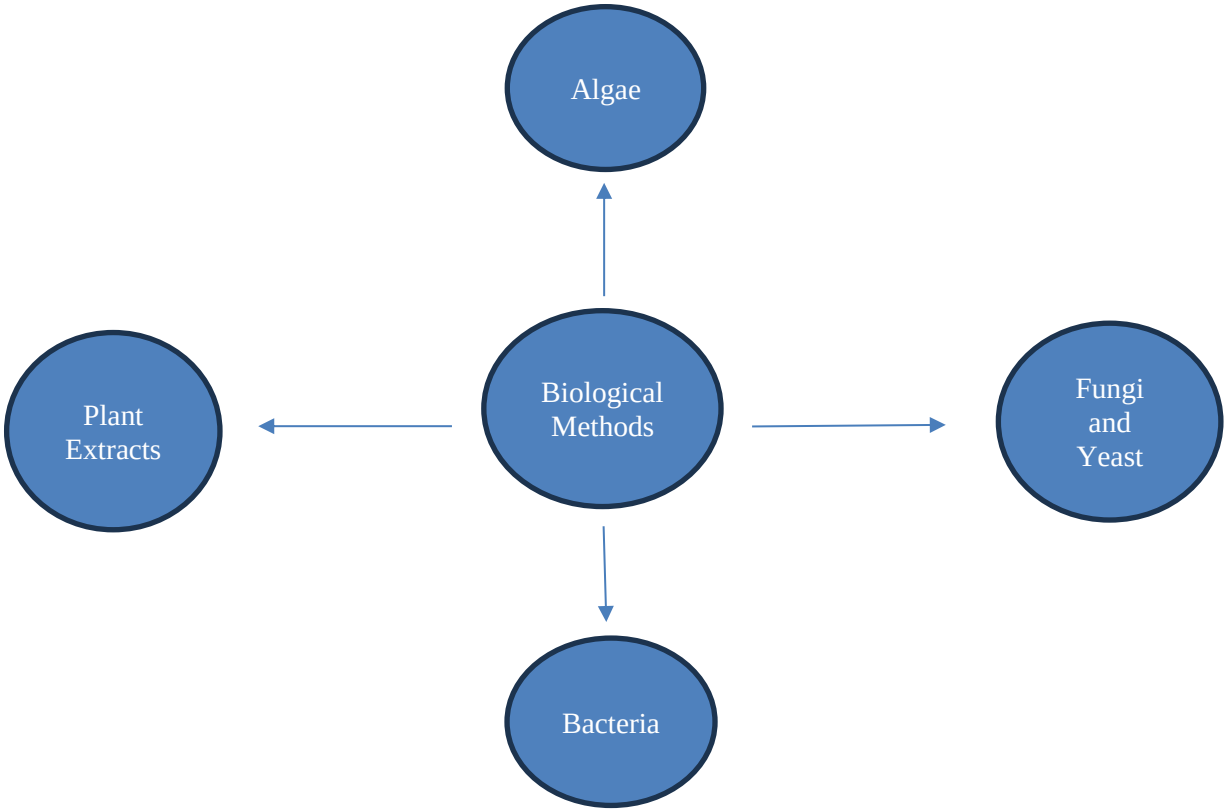


Fig 2 :- Components that can be used in green synthesis of nanoparticles[2]

Water is used as an environmentally friendly solvent throughout the preparation process in the current method. Although the manufacture of nanoparticles using different solvents, such as supercritical CO_2, has been achieved, using CO_2-philic surfactants makes it difficult to isolate and recover the nanoparticles. Additionally, there are concerns about the toxicity of the microemulsion's constituent parts. The selection of the reducing agent is the second issue with a green approach for making nanoparticles.

Reducing agents including hydrazine, sodium borohydride (NaBH4), and dimethyl formamide are used in the majority of the procedures so far reported (DMF). These are all extremely reactive substances that could be hazardous to the environment and to human health. The reducing agent in the current approach is beta-D glucose, a reducing sugar (Singh and Dutta et. al 2018)

Choosing the capping material to utilize on nanoparticles is important for their surface protection or passivation. The requisite size ranges and nanoparticle morphologies, as well as the intended application, are few of the factors that should be taken into consideration when choosing a capping agent. The protective agent that could be used is starch, which was chosen for a number of reasons.

The presence of size-constrained, nanosized pools of inter- and intramolecular origin in solutions of polymeric materials is widely known and can be utilized to create nanoparticles. For the creation of nanoparticles, both linear and dendritic polymers have been utilized successfully.

Traditional	Green approach
Complex (procedure)	Simple (procedure)
Toxic byproducts are observed	No Toxic byproducts are observed
Costly	Cheaper
High temperature	Low temperature

Tab-1 Quick comparision between traditional and green approach for synthesis of nanoparticles

Copper nanoparticles:-

CuNPs are nanosized particles of copper having special physical and chemical characteristics. They have attracted a significant amount of attention because of their potential applications in a various of fields, like chemical, biological, and environmental sciences. CuNPs can be synthesized in many ways, with physical, chemical, or biological methods offering different benefits and features. CuNPs have demonstrated potential in a number of applications, and more research is being done to explore their potential(Mahmood et. al 2018).

Various use of copper nanoparticles:-

- Food supplements containing copper with efficient delivery capabilities
- Can be used in ointments
- Efficient catalyst for chemical reactions, such as those that produce methanol and glycol
- Can be used nano-composite.
- In personal care products.
- Used in pharmaceuticals
- Can be used dental care
- Anti-fungal activities
- Anti-bacterial activities
- Can be used as surface disinfectants

.

Reasons to choose copper for the production of nanoparticles:-

- Robust
- Easily available
- Cheap
- In comparison to gold and silver it has better antimicrobial properties and activities

For instances copper nanoparticles has high antibacterial activity in comparison to silver and gold nanoparticles against E.coli and Bacillus subtilis.

Antimicrobial property of copper nanoparticles:-

Nanoparticles have unique properties due to their minuscule size. Compared to its bulk constituent, copper nanoparticles exhibit remarkable and potent anti-microbial properties due to their small size. In minuscule amounts, copper nanoparticles can exhibit antimicrobial activity better than expensive metal(Mahmood et. al 2018).

Antibacterial activity also depends on method, size, shape and concentration of NPs.

Research has shown that copper nanoparticles has effective activity against both gram (+) and gram(-) bacteria. They have been used to administer antibiotics in the fight against germs. Since many bacteria become resistant to antibiotics, copper nanoparticles can be employed as an alternative; studies and research are currently being conducted in this area (Mahmood et. al 2018). When copper is consumed in small amount it may also shows antioxidant activity. Its activity can be seen in some ways:-

- The integrity of the bacterial membrane is disrupted.
- Encourages the toxicity of copper nanoparticles which is influenced by factors such as pH, temperature, bacterial content, and aeration mechanism.
- Action on bacterial cell membrane protein.
- Denaturation of intracellular protein (RNA and DNA)

Characterization of copper nanoparticles:-

It can be done by using techniques :-

- TEM
- SEM
- XRD
- FTIR
- Cryo-TEM

Cocos nucifera (L.) (Green Coconut)

Plam tree, which generally use to grow up to 30 meters in height, which can be found in tropical regions. Its water is consumed for refreshment. Its husk and other parts can used in the treatment of diarrhea and arthritis. Green shell of coconut can be used for removal of toxic compounds from water. Can be used as oil ointment and its water can be used for hair loss. Even shell charcoal has been used for the removal of for the removal of toxic compound from water. Leishmanicidal and antimalarial properties has also been reported(Paul and Bag et.al 2014). Presence of phenolic compound can be proved by ferric chloride test. Catechins, flavonoids and other polyphenol compound has been found in lyophilized extract. Studies have also found potentially high analgesic activities and it can create path for the manufacturing of low-cost medicines. Husk fibers extract has been used on the animal (mice) it shows significant activity in proving its anti-inflammatory activities. Medium chain of fatty acids of coconut oil is been utilized for anti-viral activities and agar well diffusion method is been used for proving the antibacterial activity of alcoholic extract of husk fibers. Urogenital disorders can be treated like *Trichomonas vaginalis* (Lima and Souse et.al 2015)

Composition	Weight%
Lignin	29.4
pentosans	27.7
Cellulose	26.6
Moisture	8
Solvent Extractive	4.2
Uronic Anhydrides	3.5
Ash	0.6

Tab 2 :- Composition of *Cocos nucifera* (L.) in terms of weight percentage-wise [2]

Ethanol extract reveals :-

- Phenol
- Alkaloids
- Flavonoid
- Triterpenes
- Tannis

On the other hand its butanol extract reveals

- Saponins
- Condensed

Compunds	Coconut parts
Vitamin C	Liquid albumen
Lauric Acid	Coconut Oil
L-arginine	Liquid albumen and solid albumen
a- Tocopherol	Coconut oil
saponin	Roots
Catechin	Coconut Fiber
Flavonoid	Coconut fiber,Root and Inflorescence
Tannis	Coconut fiber and Inflorescence
Lupeol-methylether	Leaves
skimmiwallin	Leaves
Isoskimmiwallin	Leaves

Tab 3 :- Phytochemical found in Cocos nucifera linn [3]

CHAPTER 3

Material and Methods

Equipment and chemical used:-

- Pipette
- Pipette tips
- Falcon tubes
- Flask
- Beaker (1000 ml)
- UV-Visible Spectrophotometer
- Digital weight balance
- Cuvette
- Filter paper
- Water bath
- 96-well plate
- Petri dish
- Micro vials
- Test tubes
- Measuring cylinder
- Falcon stand
- Cupric sulphate ($CuSo_4$)
- *Cocos nucifera* (L.) extract
- DPPH
- Gallic acid
- Mueller hinton Agar
- Mueller hinton broth
- Agar Agar technical

Methodology for preparing the extract

One green coconut was taken and its skin was peeled off and chopped into small pieces

After peeling and chopping was done, glassware was autoclaved.

250 grams of green shell was weighed and 900 ml of distilled water was taken in beaker of 1L

Boiled on heating plate for 30 minutes and a colour change was observed in distilled water

After that solution was left to be cooled and with the help of whatt's man filter paper extract was filtered.

After filtering the extract, it was stored at 4^{o} C in cold room

With the help of spectrophotometer, reading was taken, range :- 200-800 nm. After diluting sample to 3 times.

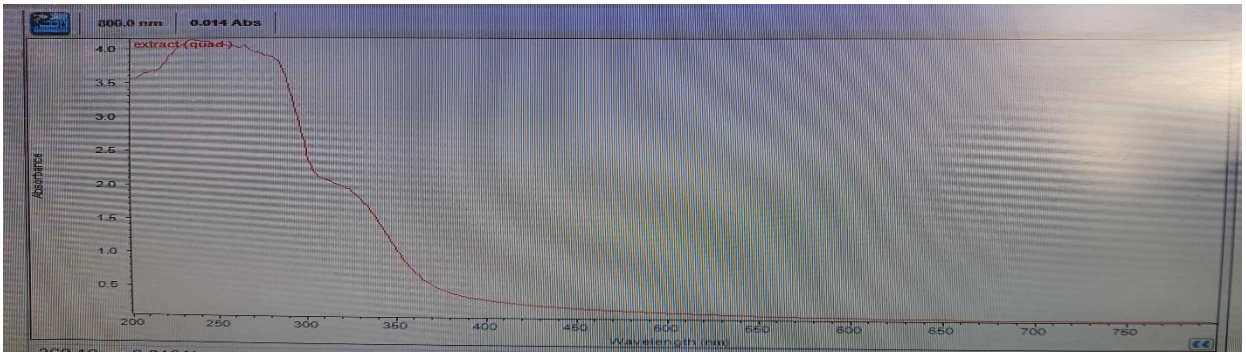


Fig-3 Spectrophotometer reading of *Cocos nucifera* (L.) green shell extract

Fig 4 Weighing of husk up to 250g

Fig 5 Heating on hot plate up to 30minutes

Fig 6 Use of cone and filter paper (125mm) bottle of 500ml extract was filtered

Fig 7 Keping at 4°C borosil

Fig 8 Growth of fungus was observed in extract if not kept at

Optimizing parameters for copper nanoparticle synthesis

Optimizing – Ratio

8 falcon tubes were taken

In each falcon tubes 10 ml of copper sulphate solution(1mm) were taken

Then in each falcon tubes with varying ratio of extract were poured (1:0.25, 1:0.5, 1:0.75, 1:1, 1:1.25, 1:1.5, 1:1.75 ,1:2) where total was kept 20ml.

For maintaining pH, pH meter was used and it was kept at 11 pH

After that nanoparticle solution was poured into test tubes and then it was kept in water bath for 20 minutes at 40℃

With the help of UV-spectrophotometer, reading was taken, range :- 200-800 nm. After diluting sample to 3 times

Optimizing parameters for copper nanoparticle synthesis

Optimizing – pH

9 falcon tubes were taken

In each falcon tubes 10 ml of extract were taken

Then in each falcon tubes copper sulphate solution (1mm) were taken in the ratio of 1:1 where 10ml is extract and 10 ml is copper sulphate solution

For maintaining pH, pH meter was used and it is maintained at 4 pH, 5 pH, 6 pH, 7 pH, 8 pH, 9 pH, 10 pH, 11 pH, 12 pH

After that solutions were poured into test tubes and then it was kept in water bath for 20 minutes at 40℃

With the help of UV-spectrophotometer, reading was taken, range :- 200-800 nm. After diluting sample to 3 times

Optimizing parameters for copper nanoparticle synthesis

Optimizing – Temperature

6 falcon tubes were taken

In each falcon tubes 10 ml of extract were taken

Then in each falcon tubes copper sulphate solution (1mm) were taken in the ratio of 1:1 where 10ml is extract and 10 ml is copper sulphate solution

.

For maintaining pH, pH meter was used and it was kept at 11 pH

After that solutions were poured into test tubes and then it was kept in water bath for 20 minutes at 30℃ 40℃, 50℃, 60℃, 70℃ and one sample was kept at 40℃ on magnetic stirrer with magnetic beads in it

With the help of UV-spectrophotometer, reading was taken, range :- 200-800 nm. After diluting sample to 3 times

Optimizing parameters for copper nanoparticle synthesis

Optimizing – Time

6 falcon tubes were taken

In each falcon tubes 10 ml of extract were taken

Then in each falcon tubes copper sulphate solution (1mm) were taken in the ratio of 1:1 where 10ml is extract and 10 ml is copper sulphate solution

.

For maintaining pH, pH meter was used and it was kept at 11 pH

After that solutions were poured into test tubes and then it was kept in water bath for 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes at 40℃

With the help of UV-spectrophotometer, reading was taken, range :- 200-800 nm. After diluting sample to 3 times

DPPH ASSAY

For observing antioxidant activity

DPPH solution is made by using 80% ethanol and incubated for 24 hours
(5 mg in 100 ml of ethanol)

Set the OD of DPPH

After setting the absorbance of the DPPH, take 12 test tubes for NPs solution, extract, copper solution and gallic acid

Pour 3 ml of DPPH in each test tube and then add 100 µl, 200 µl, 300 µl of sample in it and pour gallic acid in last 3 test tubes

Keep the test tubes for 30 minutes of incubation and observe the color change

With the help of spectrophotometer take OD of the samples and OD is taken at 517 nm

ANTI-MICROBIAL TEST

For observing inhibition zones

Revival of E.coli culture and S.aureus, one day before performing of AST

After that OD of both the culture has been checked

Prepare MHA media in a 500 ml flask and autoclave it

On next day, pouring of MHA media is done on petri plates and streaking was done

Wells were punches according to the no. of samples

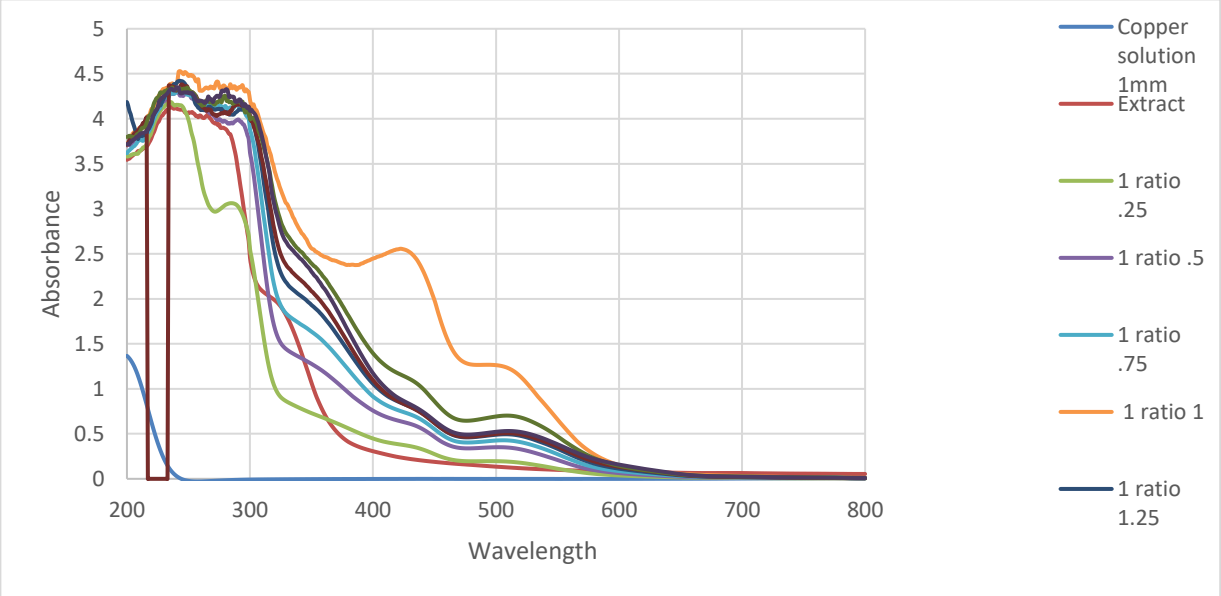
Formulations are made according to the working solution

Adequate amount of formulations were poured and result were observed on next day

CHAPTER 4

RESULTS AND DISCUSSION

ABSORBANCE VS WAVELENGTH GRAPH OF COPPER SULPHATE NANOPARTICLES WITH VARYING RATIO OF EXTRACT

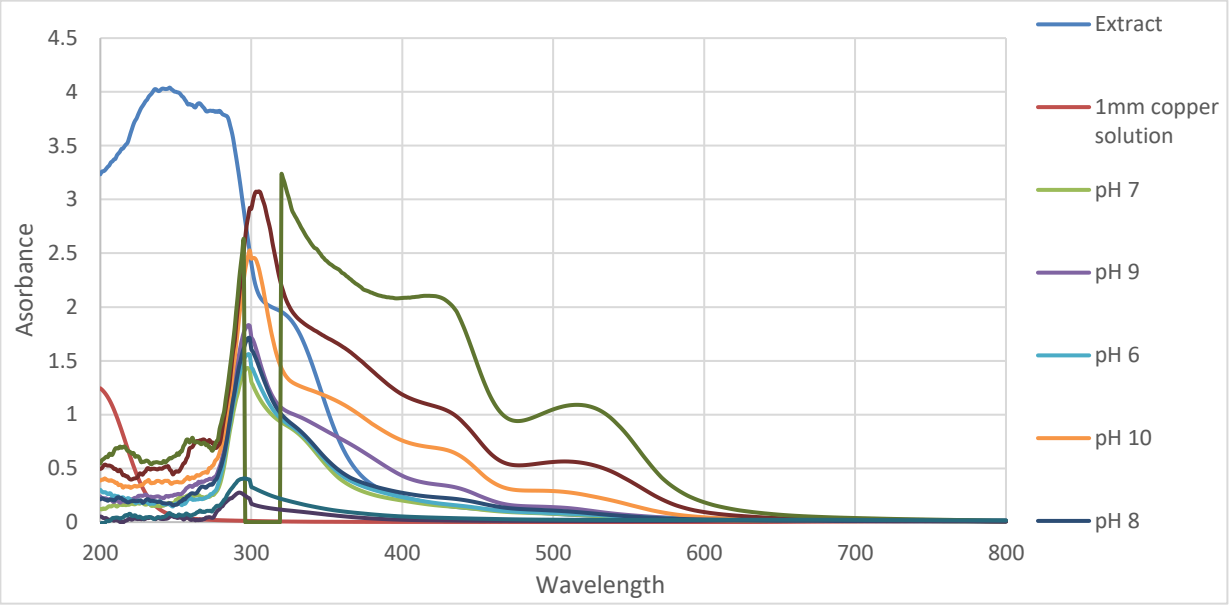


Graph-1 Graphical depiction of absorbance vs wavelength at varying ratio

Copper nanoparticles has the significant efficiency to absorb and then scatter it. Main reason behind it can be surface plasmon resonance which is generally exhibited by nano sized particles not by the bulk material. In simple words, interaction of light with the free electrons presents on the surface of metal. Also scattering of light is also dependent on the shape and size of the particle like small particles have narrow peaks and vice-versa.

Here in the above graph, it is observed that the highest peak in varying ratios is 1:1, that directly indicates the formation of maximum nanoparticles.

ABSORBANCE VS WAVELENGTH GRAPH OF COPPER SULPHATE NANOPARTICLES WITH VARYING pH

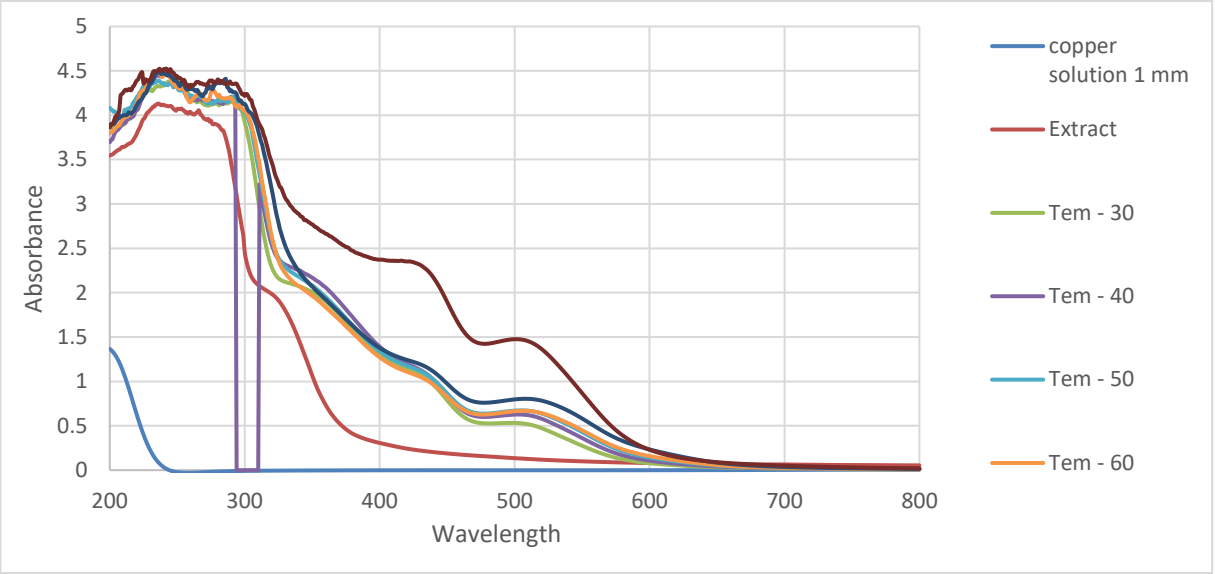


Graph -2 Graphical depiction of absorbance vs wavelength at varying pH

Copper nanoparticles has the significant efficiency to absorb and then scatter it. Main reason behind it can be surface plasmon resonance which is generally exhibited by nano sized particles not by the bulk material. In simple words, interaction of light with the free electrons presents on the surface of metal. Also scattering of light is also dependent on the shape and size of the particle like small particles have narrow peaks and vice-versa.

Here in the above graph, it is observed that the highest peak in varying pH 12, that directly indicates the formation of maximum nanoparticles, but 11 pH was selected for the synthesis of copper nanoparticles with green coconut extract via green synthesis.

ABSORBANCE VS WAVELENGTH GRAPH OF COPPER SULPHATE NANOPARTICLES WITH VARYING TEMPERATURE

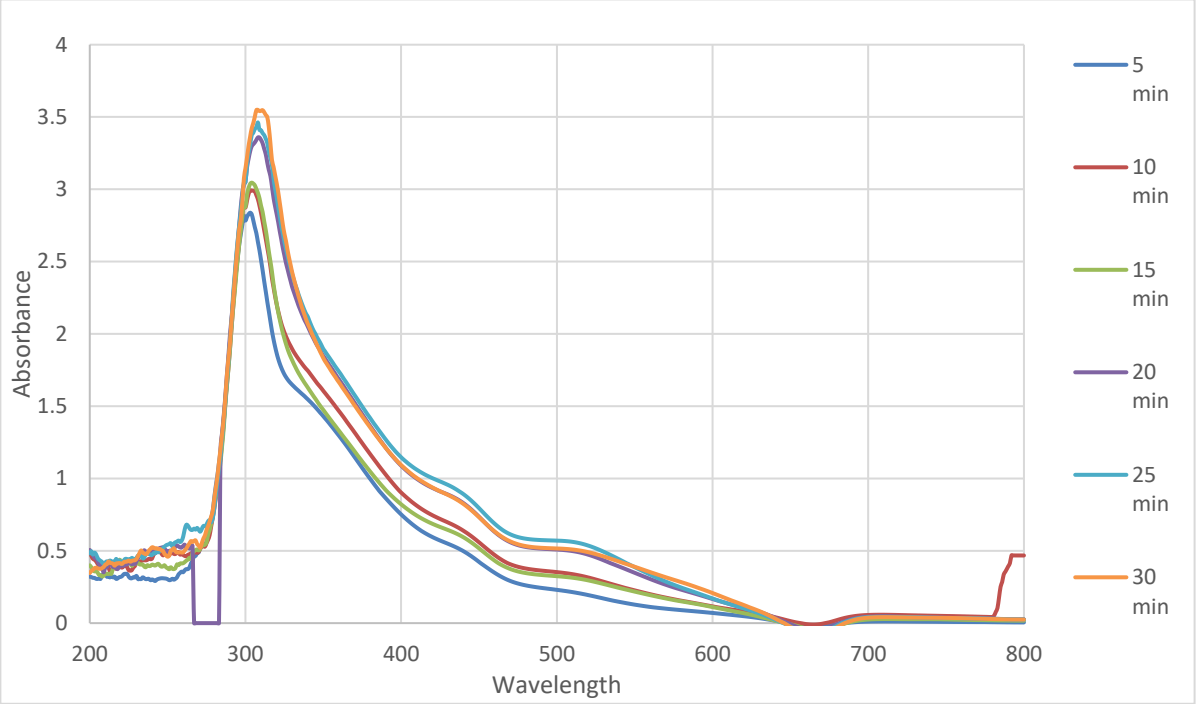


Graph 3 -Graphical depiction of absorbance vs wavelength at varying Temperature

Copper nanoparticles has the significant efficiency to absorb and then scatter it. Main reason behind it can be surface plasmon resonance which is generally exhibited by nano sized particles not by the bulk material. In simple words, interaction of light with the free electrons presents on the surface of metal. Also scattering of light is also dependent on the shape and size of the particle like small particles have narrow peaks and vice-versa.

Here in the above graph, it is observed that the highest peak in varying temperature is 40℃ stirred with magnetic bead on a hot plate, that directly indicates the formation of maximum nanoparticles, but 40℃ without stirring was selected for the synthesis of copper nanoparticles with green coconut extract via green synthesis.

ABSORBANCE VS WAVELENGTH GRAPH OF COPPER SULPHATE NANOPARTICLES WITH VARYING TEMPERATURE



Graph 4 -Graphical depiction of absorbance vs wavelength at varying time

Copper nanoparticles has the significant efficiency to absorb and then scatter it. Main reason behind it can be surface plasmon resonance which is generally exhibited by nano sized particles not by the bulk material. In simple words, interaction of light with the free electrons presents on the surface of metal. Also scattering of light is also dependent on the shape and size of the particle like small particles have narrow peaks and vice-versa.

Here in the above graph, it is observed that the highest peak in varying time is 20 minutes, that directly indicates the formation of maximum nanoparticles.

ANTIOXIDANT ACTIVITY TESTED BY USING DPPH

Fig 9 Copper nanoparticles, copper sulphate solution and extract antioxidant results

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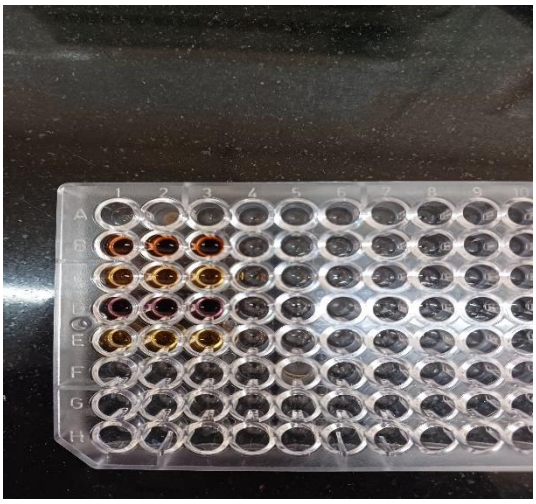
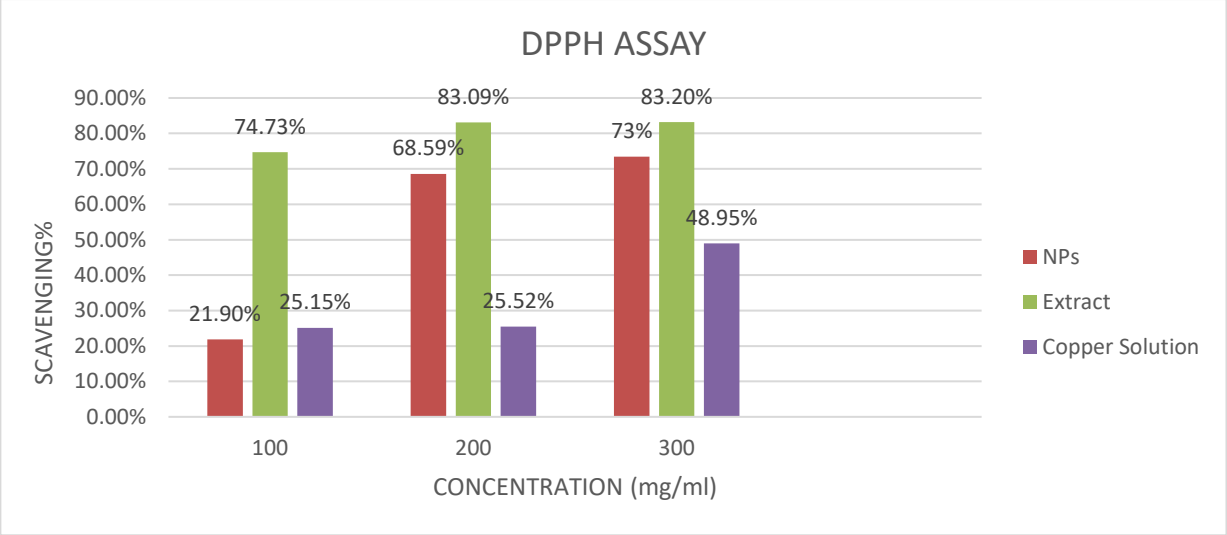


Fig 10 Loading of samples in 96-well plate

BAR GRAPH REPRESENTATION OF THE ANTIOXIDANT ACTIVITY



Graph -5 Scavenging percentage of the copper nanoparticles, extract and copper sulphate solution

Formula:-

Scavenging activity (%) = 1 – (Absorbance of sample) / (Absorbance of control) * 100

DPPH test is generally used for the purpose of measuring antiradical power of either pure molecules or plant extract. It measures the antioxidant power of a sample to reduce the DPPH by transferring of hydrogen, which initially observed as violet after reduction change into yellow color.

Here in the above graph, the highest antioxidant activity is shown by plant extract (green coconut). There are some errors the graph as it should be NPs formulation to show high antioxidant activity.

ANTIMICROBIAL ACTIVITY (MHA TEST)

Antimicrobial activity was checked by preparing the stock cultures Staphylococcus *aureus* and Escherichia *coli.*

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Fig 11 MHA test results on E.coli

Fig 12 MHA test results on S.aureus

CHAPTER 5

CONCLUSION

These days, nanobiotechnology is becoming more valuable due to its capacity to reduce metals to nanoscale sizes, whereupon they manifest distinct characteristics and can be used in different biological fields. When contrasted to their bulk counterparts, nanoparticles have distinct visual, chemical, and physical characteristics. Additionally, their area of coverage to volume ratio is large. Nanoparticles have many uses because of these characteristics. Copper nanoparticles is being used from ancient times and it has significant clinical use like in treating legionella. In some instances, copper nanoparticles proved better than other expensive metal, iron and zinc. They find use in the biomedical, food, pharmaceutical, textile, medication delivery, and pesticide industries, among many other sectors. Green coconut has been used in the food industry on a large and it produces large amount of biological waste and thus cause pollution. It has tremendous amount of potential as it has pharmacological benefits and also used in the medical field for the treatment, further by making nanoparticles from it can huge opportunities in the cosmetic and skin care industry.

Here in this case, antioxidant activity of extract is higher than that of NPs, generally it is seen that the antioxidant activity of NPs is higher than that of extract. There can be a reason behind it, phytochemical which is present in the extract may be less potent to convert the precursor of copper into copper nanoparticles.

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