SYNTHESIS OF COPPER NANOPARTICLES FROM POLYALTHIA LONGIFOLIA LEAF AQUEOUS EXTRACT AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Nanotechnology and Nano particles based product and application are increased now a days due to the biological effectiveness . The scope of the present study is to synthesize copper Nanoparticles and evaluate its antibacterial activity with Polyalthia longifolia extract. The objectiveof the present study is to investigate the green synthesis method used to produce the Nanoparticles. Polyalthia longifoliais an evergreen shrub or small tree in the dogbane family of Apocynaceae, toxic in all its parts. It is theonly species currently classified in the genus Polyalthia. The leaf extract of Polyalthia longifoliais used for the synthesis of Copper Nanoparticles. The scope of the present study is to synthesize copper nanoparticles and evaluate its antibacterial activity. The present study deals with synthesis ofcopper Nanoparticles and characterization of synthesized copper Nanoparticles confirmed by UV-visible spectrophotometer, FTIR analysis and also the analysis ofantimicrobial activity of copper nanoparticles.

Keywords: Antimicrobial, FTIR, Copper nanoparticles, Polyalthia longifolia, UV-spectrometer.

1.INTRODUCTION

In recent years, Nanotechnology has attracted many researchers from various fields like biotechnology, physics, chemistry, material sciences, engineering and medicine. Nano particles are synthesized by physical and chemical Methods andthese are suffering from drawbacks like expensive

reagent, hazardous reaction condition, longer time, tedious process to isolate nano particles. Hence, there is a scope to develop new methods for the synthesis of nano particles which should be required inexpensive reagent,less drastic reaction condition and eco-friendly. In recent years, Copper Nano particles have attracted much attention of researchers due to its application in wound dressings and biocidal properties, potential industrial use such as gas sensors, catalytic process, high temperatures super conductors. Polyalthia longifolia, the Ashoka native to India, is a lofty evergreen tree, commonly planted due to its effectiveness in alleviating noise pollution. It exhibits symmetrical pyramidal growth with willowy weeping pendulous branches and long narrow lanceolate leaves with undulate margins. The tree is known to grow over 30 ft in height. Polyalthia is derived from a combination of Greek words meaning 'many cures' with reference to the medicinal properties of the tree while Longifolia, in Latin, refers to the length of its leaves. Green synthesis of copper Nano particles was achieved by using microorganisms and plantextract.

2.SCOPE AND OBJECTIVES

The scope of the present study is to synthesize copper Nanoparticles and evaluate its antibacterial activity in polyalthia longifolia extract.The objectives of the present study is to investigate synthesis of copper Nanoparticles and to characterize the synthesized copper Nanoparticles in polyalthia longifolia extract and confirmed by UV-visible spectrophotometer and FTIR analysis.

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL

The powder of Polyalthia longifolia leaf was weighed 5g and dissolved in 100ml of distilled water and boiled for 20 min at 50^0 C. The extract was filtered by Whatmann No1 filter Paper. Then the filtrate was stored in a tight seal pack under 4^0 C for further use. The reaction mixture was prepared by adding 80ml of 1mM $Cuso_4$ and 20ml of Polyalthia longifolia leaf Extract. Blank was prepared by adding 80ml of deionised water to 20ml of Polyalthia longifolia leaf extract. The formation of copper Nanoparticles (reduction of Cu^{2+} to Cu^{+} ion) was indicated by color change from light color to dark color. The addition of a certain amount of Cu^{2+} can effectively inhibit Cu^{2+} reduction into Cu^{+} during the colour changes. The conversion is act as a reversible one from the Cu^{+} to Cu^{2+} with help of electron transfer from reactant side to product side.

3.2 UV-VISIBLE SPECTROPHOTOMETER ANALYSIS

The synthesized copper Nanoparticles were characterized using UV-Vis spectrophotometer HITACHI U2300. The formation of copper Nanoparticles was Monitored by UV spectrophotometer in the range of absorbance from 250-480nm.

3.3 FOURIER TRANSFORM INFRARED SPECTROSCOPY [FTIR]

The reaction mixture containing Copper Nonoparticles prepared from Polyalthia longifolia leaf extract was poured into a petridish and kept in a hot air oven until it was getting dried off. After that the dried sample was scrubbed and the powder form of sample is stored in a sterile eppendorf. Then it was used for the FT-IR analysis in theregion of 400-4000cm^{-1}.

3.4 PREPARATION OF REACTION MEDIUM FOR THE ANALYSIS OF ANTIBACTERIAL ACTIVITY

Two different bacteria (Escherichia coli, Staphylococcus aureus) were taken from the stock cultureand dissolved in 25 ml of Nutrient bothand kept for 12 hrs incubation. The synthesizedcopper nanoparticles using Polyalthia longifolialeaf extract was tested for its antibacterialactivity by agar well diffusion methodagainst Escherichia coli and Staphylococcus aureus.

4. RESULTS AND DISCUSSIONS

4.1 ULTRAVIOLET/VISIBLE (UV/VIS) SPECTROSCOPY ANALYSIS

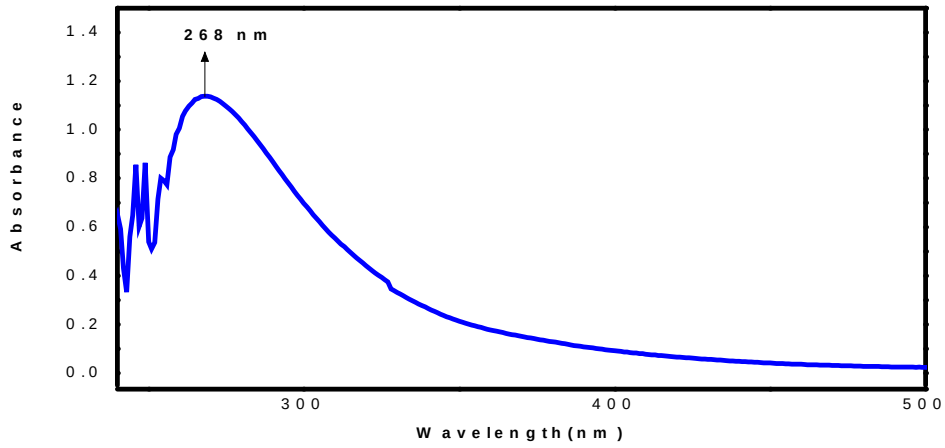

Figure 1 : ULTRAVIOLET VISIBLE SPECTROSCOPY OBTAINED FOR COPPER NANOPARTICLES

The reduction of Cu+ ions was monitored by UV- Spectrophotometer. The characterization of copper Nanoparticles in the plant extract was done by with help of UV-Spectrophotometer. The standard peak value of copper nanoparticle in UV 250-450nm[1] . Figure 1, shows that the broad peak obtained at 267- 268 nm. The peak confirms the presence of copper nanoparticle in the Polyalthia longifolia leaf extract.

4.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

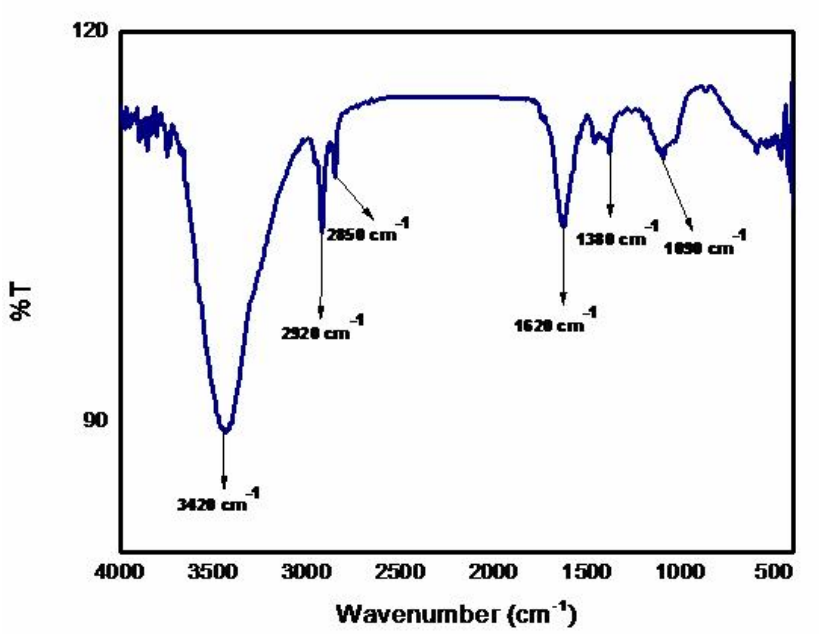

Figure 2 : FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) OBTAINED COPPER NANOPARTICLES

The FT-IR Characterization is used to find the molecules and their functional group present in the synthesized copper Nanoparticles. Figure 2, represents the FT-IR spectra peaks at 3420cm^{-1}, 2920cm^{-1}, 2850cm^{-1}, 1650cm^{-1}, 1380cm-1, and 1090c cm^{-1} [2]. TheFTIR spectra revealed the presence ofdifferent functional groups like Alcohol (OH stretch H-bonded, free), Alkane (C-H stretch, -C-H bending)) Alkene (=C-H bending, C=C stretch) ,Amine (C-N, Strech) Nitro compounds (N-O stretch), Acid (OH, stretch) Ester, (C-O, stretch). These functional groups play a very important role in copper nanoparticle synthesis.The region of copper nanopartical in FT-IR is 400 – 4000 cm^{-1}. Different peaks obtained in FTIR specrtum confirmed the presence of copper nanoparticals in plant extract.

4.3 ANALYSIS OF ANTIMICROBIAL ACTIVITY

TABLE 1 : ANTIMICROBIAL ACTIVITY OF COPPER NANO PARTICLE

MICROORGANISM	PLANT EXTRACT WITHOUT COPPER NANO PARTICLES (30µl)	PLANT EXTRACT WITH COPPER NANO PARTICLES (30µl)	CHLOROMPHENICAL (30µl) (STANDARD)
SIZE OF ESCHERICHIA COLI (in mm)	9	10	18
SIZE OF STAPHYLOCOCCUS AUERUS (in mm)	9	10	20

Figure 3 : ANTIMICROBIAL ACTIVITY OF COPPER NANO PARTICLE

Table : 1 and Fig : 3 shows that the standard size of Escherichia coli and Staphylococcus are 18 and 20 in chloromphenical (STD). In blank solution, the size of Escherichia coli and Staphylococcus are 9. In the plant extract solution with Copper nano particles ,the size increase from 9 to 10 for both Escherichia coli and Staphylococcus and it is lesser than the standard size in chloromphenical.

5. CONCLUSION

The aqueous copper ions exposed to the Polyalthia longifolialeaf extract results in the formation of copper nanoparticles was confirmed by the change of colour. Synthesized copper nanoparticles was confirmed by UV Visible spectrum and FTIR specturm. Proven antibacterial activity against different microorganisms such as E. coli and S. aureus established. It is confirmed that the copper nanoparticals are capable of rendering high antibacterial efficacy and hence it has a great potential in the preparation of drugs used against bacterial diseases. Copper nanoparticles based on these findings leads to valuable discoveries in various fields such as medical devices and antimicrobial systems.The present study exhibit a simple method of synthesis of copper nanoparticles and it is used for industrial production of nanoparticles at room temperature with more potent antimicrobial agents.

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NANOTECHNOLOGY - APPLIED IN MEDICINE AND BIOLOGICAL PROCESS

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ABSTRACT

The article is about the various applications of nanoparticles in medicine and biology. Nanoparticles are metal particles in the size range of 1-100nm and form building blocks of nanotechnology. Nanotechnology is a multidisciplinary science comprising various aspects of research and technology. Metal nanoparticles like gold, silver and platinum have gained considerable attention in recent times due to a wide variety of potential applications in biomedical, optical and electronicfields. The wide range of applications of nanoparticles is due to their unique optical, thermal, electrical, chemical and physical properties that are due to a combination of the large proportion of high energy surface atoms compared to the bulk solid. Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures.

Their unique size-dependent properties make these materials superior and indispensable in many areas of human activity. This brief review reveals the most recent developments in the field of applied nanomaterials, in particular their application in biology and medicine, drug delivery, imaging, sensing, and for the understanding of basic biological processes.

1. INTRODUCTION

Nanotechnology is a relatively new branch of science that has found a wide range of applications that range from energy production to industrial production processes. One of the key applications of nanotechnology is in field of biology and biomedical research. Nanoparticles (NPs) can be engineered to possess unique composition and functionalities, which can provide novel tools and techniques in biomedical research (1).

2. TYPES OF NANOPARTICLES

There are many types of NP platforms with differing size, shape, compositions, and functionalities. The major characteristics and functionalities of each NP that is relevant for biomedical research.

I. LIPOSOMES

The first NP platform was the liposomes. It is one of the first NP platforms to be applied for gene and drug delivery. Liposomes are spherical vesicles that contain a single or multiple bilayered structures of lipids that self-assemble in aqueous systems. Unique advantages imparted by liposomes are their diverse range of compositions, abilities to carry and protect many types of biomolecules, as well as their biocompatibility and biodegradability (2). These advantages have led to the well-characterized and wide use of liposomes as transfection agents of genetic material into cells (lipofection) in biology research. Another major application of liposomes is their use as therapeutic carriers since their design can allow for entrapment of hydrophilic compounds within the core and hydrophobic drugs in the lipid bilayer itself (3). Today, there are twelve clinically approved liposome-based therapeutic drugs.

II. ALBUMIN-BOUND NPs

Albumin-bound NPs (nab) uses the endogenous albumin pathways to carry hydrophobic molecules in the bloodstream. Albumin naturally binds to the hydrophobic molecules with non-covalent reversible binding, avoiding solvent-based toxicities for therapeutics. As a result, this platform has been successfully adapted as drug delivery vehicle. Abraxane, a 130-nm nab paclitaxel was approved by the FDA in 2005 for the treatment of metastatic breast cancer (4). It may also target the albumin-binding protein SPARC (Secreted Protein Acidic and Rich in Cysteine), which is over expressed in certain tumors.

III. POLYMERIC NPs

Polymeric NPs formed from biocompatible and biodegradable polymers have been extensively investigated as therapeutic carriers. Polymeric NPs have been formulated to encapsulate hydrophilic and/or hydrophobic small drug molecules, as well proteins and nucleic acid macromolecules. The NP design can allow for slow and controlled release of drug at target sites (5). Polymeric NPs are usually able to improve the safety and efficacy of the drugs they carry. Another type of polymeric NP is dendrimers. Dendrimers are regularly branched macromolecules made from synthetic or natural elements including amino acids, sugars, and nucleotides. The varied combination of these components can yield dendrimers of well-defined size, shape, and branching length/density. As a result of their unique design, dendrimers can be developed as sensors as well as drug and gene delivery carriers (6).

IV. IRON OXIDE NPs

Iron oxide NPs are widely studied as a passive and active targeting imaging agent as they are mainly superparamagnetic. Currently, two SPIO agents, ferumoxides (120–180 nm) and ferucarbotran (60 nm) are clinically approved for MRI (Magnetic Resonance Imaging). SPIONs (Superparamagnetic iron oxide NPs) have also been used in molecular magnetic resonance applications such as the detection of apoptosis and gene expression. SPIONs can be functionalized with magnetic, optical, radionuclide and specific targeting ligands for multimodal imaging (7). They can also potentially be used as non-invasive diagnostic tools and as drug delivery vehicles.

V. QUANTUM DOT

First discovered in 1980, quantum dots (QDs) are semiconductor particles that are less than 10 nm in diameter. QDs display unique size-dependent electronic and optical properties. Most QDs studied consist of a cadmium selenide (CdSe) core and a zinc selenide (ZnS) cap (8). The absorption spectra of these particles are very broad and emission is confined to a narrow band. QDs can also emit bright colors, have long lifetimes, high efficiencies and are stable against photo bleaching. They can be generated to have different biochemical specificities and can be simultaneously excited and detected. As a result, QDs have several significant advantages over many organic fluorophore dyes for optical applications.

VI. GOLD NPs

Gold NPs offer many size-and-shape dependent optical and chemical properties, biocompatibility, and facile surface modification. Gold NPs can strongly enhance optical processes such as light absorption, scattering, fluorescence, and surface-enhanced Raman scattering (SERS) due to the unique interaction of the free electrons in the NP with light (9). These properties have enabled the realization of gold NPs in many applications such as biochemical sensing and detection, biological imaging, diagnostics, and therapeutic applications. Gold NP probes have also been used to detect heart disease and cancer biomarkers (10). They can also transform absorbed light into heat and therefore, have high potential for infrared phototherapy.

3. NANOPARTICLE APPLICATIONS IN BIOLOGY

The various applications of nanomaterials in biology or medicine are:

I. NANOPARTICLES FOR PATHOGEN DETECTION AND SEPARATION

Various NP platforms have been explored as sensors for detection and separation of pathogens. One of the most common methods used for the detection of bacteria has been through the use of magnetic biosensors that involve direct immunological reactions using magnetic NPs coated with antibodies against surface antigens. One group applied this immunomagnetic approach in a novel microfluidic device to attract molecules bound to magnetic NPs from one laminar flow path to another *via* a local magnetic field gradient (11). Using this device, E. coli labeled with biotinylated anti-E.coli antibody and bound to streptavidin-coated SPIONs were efficiently separated from solutions containing densities of red blood cells similar to blood.

II. NANOPARTICLES AS SENSORS

NPs have been employed in sensors for a variety of applications including detecting analytes at very low concentrations, detecting and separating pathogens, detecting and capturing cells, and detecting molecular and cellular functions.

III. NANOPARTICLES FOR ANALYTE DETECTION

The development of new sensing techniques for biological analytes such as DNA, RNA, and proteins are leading to more sensitive and efficient detection of these analytes at low concentrations. NPs have large surface area to mass ratio, small size, and composition dependent properties that can enable the use of surface ligands as a way to amplify the detection threshold or provide more rapid detection (12). The ability to easily functionalize NPs with targeting ligands can also enable specificity in binding and signaling of the analytes, allowing for more efficient detection.

IV. NANOPARTICLES FOR CELL DETECTION AND SEPARATION

NPs have been explored as sensitive tools for the detection of specific cell types and cells found in low frequency. One application of interest has been the detection and capture of circulating tumor cells (CTCs). CTCs can aid in the understanding of the biology of cancer metastasis and have been described as a strong prognostic biomarker for overall survival in patients with metastatic breast, colorectal, and prostate cancer. These techniques involve the use of magnetic NPs to target and isolate CTCs using a ligand-receptor based mechanism.

V. NANOPARTICLES AS IMAGING AGENTS

NPs have been explored as novel labels and contrast agents in molecular imaging. The unique properties of NPs can enable sensitive and specific monitoring of molecular targets as well as of cell responses associated with diseases such as cancer and cardiovascular diseases (13)

VI. NANOPARTICLES FOR TARGETED IMAGING

NPs have many promising attributes for targeted imaging. First, NPs can deliver a large number of imaging agents at a time due to their surface area, allowing for improvement in sensitivity (14). NPs may passively target tissues *in vivo* via the EPR effect or be targeted to accumulate at sites where the molecular target is expressed, increasing the local concentration of contrast agents. The high capacity for NP modification enables their use as amplifiers for *in vivo* imaging. Finally, they can deliver several different types of imaging agents to perform multimodality imaging.

4. NANOPARTICLES AS DELIVERY VEHICLES

I. DELIVERING SIRNA FOR BIOLOGICAL STUDIES

RNA interference (RNAi) is an important biological tool for use in cell culture and in living organisms. It is traditionally used to study gene functions because it allows targeted degradation of mRNA after the introduction of sequence-specific double stranded RNAs into cells. However, effective siRNA delivery can require overcoming many biological obstacles: 1) difficulty entering the cell because of high molecular weight and negative charges, 2) degradation by nucleases within the cell, 3) targeting to the appropriate cell compartment and 4) rapid clearance and instability *in vivo* (15). Recently, NPs have been used to deliver siRNA to silence genes in immune cells since these cells can have pivotal roles in homeostasis and disease. NPs have also been used as a vehicle to deliver siRNA in plant cells to study cellular pathways at the single cell level.

II. DELIVERING HYDROPHOBIC COMPOUNDS WITHOUT SOLVENT OR EXCIPIENTS

Many biologically active compounds are hydrophobic molecules and are poorly soluble in water. Utilizing such compounds in biological research can be challenging because of their poor solubility in aqueous environments. Current approaches involving using a solvent such as dimethyl sulfoxide (DMSO) or an excipient such as cremophor.

For example, wortmannin, a PI3 kinase inhibitor and a commonly utilized reagent in biological research, requires DMSO for *in vitro* applications. Moreover, NP wortmannin functioned as an effective and potent therapeutic agent *in vivo* in a mouse model of cancer.

III. DELIVERING AGENTS TO SUB CELLULAR ORGANELLES

One area of active investigation is delivering various agents to specific organelles. In particular, subcellular availability and accessibility of a target is important in effective delivery of therapeutic and imaging agents (16). Targeted NPs can bind to targets localized on the cell surface and enter the cell through endocytosis. In particular, NPs carrying oligonucleotides need to escape the endosome and then be targeted to be effective. Tools for effective subcellular targeting are emerging for targeted delivery to the nucleus, cytosol, mitochondria, endosomes, and lysosomes(17).

IV. EFFECTS OF BIOLOGY ON NPs

Understanding NP interactions with biological systems is necessary in developing effective NPs as sensing, imaging and drug delivery agents. Properties of NPs such as size, shape, functional groups, surface charge, and composition are all factors that have been shown to affect NP interactions with biological systems *in vitro* and*in vivo*. It is known that NPs are rapidly cleared by the cells of the reticulo endothelial system (RES)/mononuclear phagocyte system (MPS) in the body (18).

V. NANOPARTICLES TO STUDY BIOLOGICAL PROCESSES

The unique properties of NPs have enabled their use as promising tools to study biological processes. Many innovative techniques using NPs are being developed to activate cell signaling pathways, to induce protein production, and to improve upon current techniques used in molecular and cellular biology research. NPs such as QDs have been extensively studied for many biological applications that use fluorescence. Some of its uses include immunostaining of fixed cells and tissues, membrane proteins and cytoskeleton filaments (19). Recently, QDs have also been used to visualize the molecular dynamics of individual molecules in live cells.

5. CONCLUSION

Biological studies that employ NP techniques can provide novel insights into cell functions and molecular processes involving complex signaling pathways. Nanotechnology enable the creation of devices on the same scale as individual cells and biomolecules, creating a unique approach to imaging, sensing, drug delivery and characterizing basic biological processes. NP monitoring and

detection techniques can potentially aid in understanding the basis of biochemical pathways involved in disease and injury. Currently, there are various commercial NPs available for use as contrast agents in imaging modalities such fluorescence imaging and magnetic resonance imaging and for detection of low concentrations of analytes. Thus, careful evaluation and effects induced by different types of NP formulations in biological systems are important in employing NPs for biological applications.

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