Biogenic synthesis of silver and copper nanoparticles from leaf extract of *Catharanthus roseus*: A comparative study

To be submitted as Major Project in partial fulfilment of the requirement for the degree of

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Industrial Biotechnology

Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled **"Biogenic synthesis of silver and copper nanoparticles from leaf extract of *Catharanthus roseus*: A comparative study**" submitted by Ayushi Verma (2K18/IBT/04) in partial fulfilment of the requirements for the reward of the degree of Master of Technology, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by her under my guidance. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honoring any other degree.

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DECLARATION

This is to certify that the thesis of Major Project II entitled **"Biogenic synthesis of silver and copper nanoparticles from leaf extract of *Catharanthus roseus*: A comparative study**" submitted by Ayushi Verma (2K18/IBT/04) in the partial fulfilment of the requirements for the reward of the degree of Master of Technology, Delhi Technological University (Formerly Delhi college of Engineering, University of Delhi), is an authentic record of my own work carried out under the guidance of my project supervisor ***Dr. Navneeta Bharadvaja***, Assistant Professor, Department of Biotechnology, DTU. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honouring of any other degree.

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ABSTRACT

Nano-biotechnology is trending and biogenic metallic nanoparticles have been continuously evaluated to be utilized in many industrial applications for a decade now. The properties of nano-scale material can be drastically changed when compared to its significant bulk size. Nano-scale materials are highly efficient due to increased surface area. Sometimes they exhibit certain anomaly which can be used for numerous new applications. The advance of dependable and conservationist strategies for the development of nanoparticles is elemental to the subject of nanotechnology. Green protocols approves of co-existence of environment and technology as they are environmentally benign, rapid and economical. AgNPs and CuNPs have been produced from *Catharanthus roseus* Linn and characterized for their morphology and function. Copper and silver nanoparticles prepared were characterized structurally and chemically through UV-Visible spectroscopy, SEM, FT-IR, DLS and zeta-potential. Environmental contamination by heavy metals is arising, threatening water ecosystem and soil quality today. Remediation of heavy metals is of great concern due to their intractability and their perseverance in the ecosystem. To restrict the spread of the heavy metals within water and soil, copper and silver nanoparticles were found useful with the objective of remediation of the hostile heavy elements, namely; chromium and cadmium. This work also targets at studying the antibacterial activities of metallic nanoparticles against gram positive bacteria *Staphyloccocus aureus.*

Keywords: *Catharanthus roseus*, Silver nanoparticles, Copper nanoparticles, Biogenic synthesis, Heavy metal removal, Antibacterial

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LIST OF ABBREVIATIONS

NPs	Nanoparticles
Ag	Silver
Cu	Copper
nm	Nanometer
mm	Millimetre
µm	Micrometre
mM	Millimolar
$AgNO_3$	Silver Nitrate
CuSO4	Copper Sulphate
ml	Millilitre
µl	Microlitre
mg/l	Milligrams/litre
°C	Degree Celsius
pH	Potential of Hydrogen
ppm	Parts per million
rpm	Rotation per minute
kV	Kilovolt
SEM	Scanning Electron Microscope
FT-IR	Fourier Transform Infrared Spectroscopy
DLS	Dynamic Light Scattering
UV-Vis	Ultra-violet Visible
AgNPs	Silver nanoparticles
CuNPs	Copper nanoparticles
NCL	National Chemical Laboratory

CHAPTER 1

INTRODUCTION

It is the perfect time that we started focusing on nanotechnology or nano-biotechnology specifically. We have been provided enough information on the molecular structures and functions by allied sciences. It makes perfect sense that we put this information in use for developing advanced techniques at atomic level or nano-scale level precisely.

The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom. It is not an attempt to violate any laws; it is something, in principle, that can be done; but in practice, it has not been done because we are too big.

-Richard Feynman*

Nanotechnology can be described as the capability of building and shaping matter single atom at a time. The most fortunate thing about merging biotechnology with nanotechnology is that, it is accessible even with modest resources. The properties of a nano-scale material can be drastically changed when compared to its significant bulk size. Nano-scale materials are highly efficient due to increased surface area. Sometimes they exhibit certain anomaly which can be used for numerous new applications. Nanotechnology has already become a part of biotechnology in medical field with nanomedicines and therapy. Nano-biotechnology without a doubt has a wide spectrum of gaining momentum in upcoming years.

Metallic Nanoparticles

A nanoparticle is a single basic complete entity (with regard to its properties as well as structure) of nanotechnology. Particles that range from one to hundred nanometers are described as nanoparticle. The term nanoparticle has greek origins from the word nano meaning “small” or “dwarf” (You et al.,2013). Ascribable to the extrinsic characteristics of nanoparticles, they have an ever growing response in fields of organic/inorganic chemistry, material sciences as well as medicine. Apart from this nanoparticles have high surface reactivity which makes them although more significant.

Metal nanoparticles specifically are of great importance. Most of the noble metals are converted to nano forms for utilization in disease diagnosis and polymer preparations. Metallic nanoparticles are also used for catalysis.

Nanoparticle Synthesis

Numerous methods have been engaged in the generation of metallic nanoparticles. Both physical and chemical processes play a role in this. The selection of method of synthesis is also a crucial process as nanoparticles generated from different methods can differ in their physicochemical properties, morphology and stability.

The major two methods of nanoparticle production is 1.top down approach 2.bottom up approach.

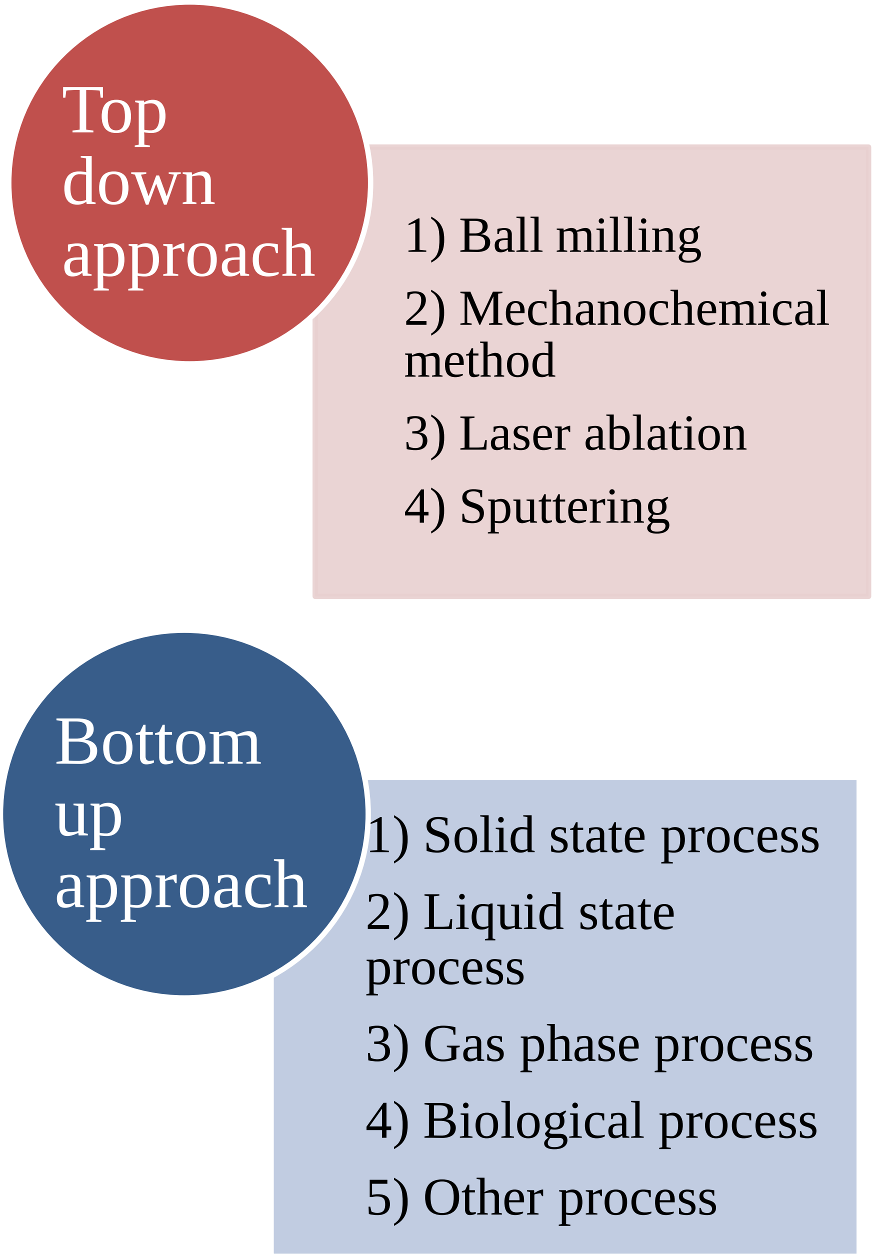


Figure 1: Approaches of nanoparticle synthesis

Examples of solid state processes are physical and chemical vapour deposition. Liquid state synthesis includes chemical reduction, hydrothermal, solvothermal and sol-gel methods. Spray pyrolysis and flame pyrolysis are gas phase process of production of nanoparticles. Other methods of nanoparticle synthesis are supercritical fluid and ultrasound processes.

Biological methods

Biological methods also called as biogenic/biomimetic route synthesis, is one of the most popular method of generating metal nanoparticles. There are a dozen of reasons for choosing green synthesis over conventional methods. Green synthesis poses no threats to the environment. High availability of raw material, low cost, low energy consumption and reduced health hazards are few benefits of this method. The infrastructure requirement is almost negligible when compared to sophisticated techniques.

The technique utilizes microorganisms, their enzymes, plant isolates and extracts generally for synthesis. During bioreduction processes microbial enzymes reduce metal ions chemically into inert nanostructures. During biosorption the metal ions can bind to cell of microbes where nanostructures are formed by peptide interaction. (Pantidos et al.,2014). Bacteria, fungus, yeast, algae and plant extracts have been used until now for the synthesis of metal nanoparticles (Yong et al.,2002)

Applications of metallic nanoparticles

Gold nanoparticles are a part of many ayurvedic medicine preparations. It has been used for boosting immunity and drug delivery purposes (Bhumkar et al.,2007). Silver naoparticles are widely known for its antimicrobial properties. Many nanoparticles such as platinum, iron and chromium have been found useful for bioremediation. Their potential for dye degradation and heavy metal removal from soil and aquatic environment has been observed. Metal nanoparticles are also used to elevate the efficiency of present technologies of remediation. Other metallic nanoparticles are anticipated for biomedical applications (Puvanakrishnan et al., 2012).

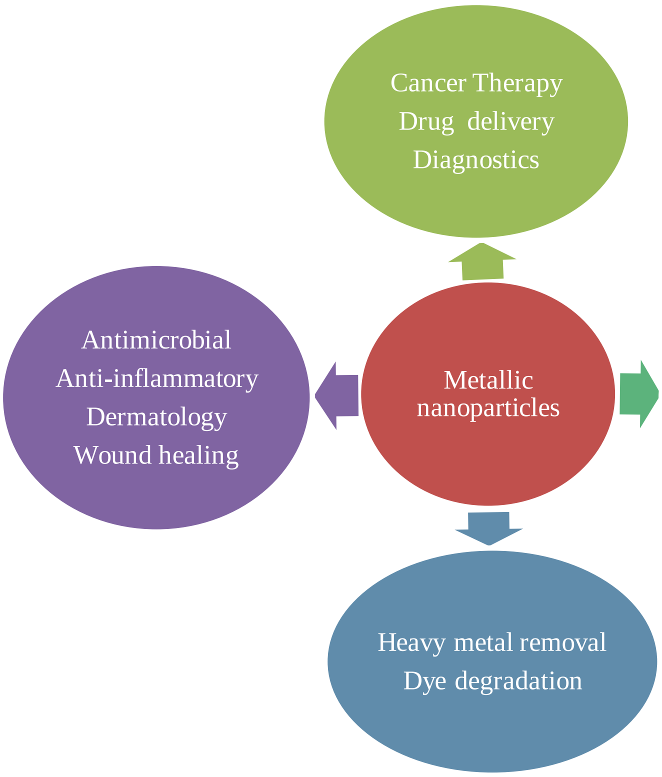


Figure 2: Diverse applications of nanoparticles

Catharanthus roseus

Catharanthus roseus also known as Madagascar periwinkle, rose prewinkle or bright eyes is an evergreen subshrub (thus called Sadabahaar in India) (Wikipedia.org). The plant is native to Madagascar but it is found in almost every tropical region.

Figure 3: *Catharanthus roseus* Linn, at DTU campus

Pharmacological activities

The plant has established itself in the ranks of medicinal plants due to its wide variety of curative properties. *Catharanthus roseus* is angiosperm, dicot plant that produces vinblastine and vincristine. The two terpene indole alkaloids play a vital role in fighting cancer (Ajaib et al., 2010). The extract from leaves in ethanol has antidiabetic effects. This has been determined from the hypoglycaemic properties of its alkaloids (Chattopadhyay et al.,1991). Plant extracts of *C. roseus* posess antimicrobial properties (Patil et al., 2010). Plant extracts also show high antioxidant, antihelmithic, anti-ulcer and wound healing properties. Vinpocetine synthesized by the plant indicates memory enhancing properties and thus can be useful in curing Alzheimer's disease (Sekar et al.,1996).

Besides the above mentioned pharmacological activities of plant extract of *Catharanthus roseus*, it can be utillized for biogenic production of metal NPs. The same process is used for the generation of AgNPs and CuNPs in the present study.

OBJECTIVES OF THE STUDY

This study majorly focuses on following points.

1. Biogenic synthesis of silver and copper nanoparticles from the leaf extracts of *Catharanthus roseus* (wild species)
2. Optimization of synthesized silver and copper nanoparticles.
3. Characterizing nanoparticles.
4. Determination of heavy metal removal potential of synthesized nanoparticles.
5. Determination of antibacterial activity of synthesized nanoparticles

CHAPTER 2

REVIEW OF LITERATURE

Spike in population, establishment of new industries every year and urbanisation all combined have disoriented the natural ecosystem of both soil and water. Untreated domestic water releases poisonous toxins into water. Nearby water bodies are the most favourable dumping sites for industrial waste. The runoff from agricultural lands rips off many harmful pesticides and fertilizers into rivers and lakes. Maximum proportion of developing countries face a tremendous water pollution crisis. Some examples of toxic pollutants which are released in water bodies and soil environment are surfactants, dyes, pharmaceutical products, pesticides and metalloids. They are highly threatening to life if consumed and may even be fatal (Abd El-Lateef et al., 2018).

Heavy metals

Heavy metals are metallic elements that have normally higher atomic density than 4000 kg/m^3 (Edelstein et al., 2018). Toxicity of heavy metals is inevitable even if they are present in traces. A few heavy metals are chromium, arsenic, zinc, mercury, molybdenum, cobalt, strontium and lead. Many of the heavy metals such as copper, iron, boron, nickel, molybdenum etc are daily requirement in plant growth. Regardless if these heavy metals accumulate beyond their permissible limit, they can cause negative impacts on plant as well as soil creatures (Hawkes et al., 1997). Other metalloids like cadmium, lead and mercury are absolutely irrelevant to plant functions and enter into the ecosystem through industrial waste, fertilizers, weathering and sewage (Liu et al., 2018). Heavy metals pollution is not limited to soil toxicity; they can accumulate within plant and disrupt their metabolic functioning. This can lead to abrupt loss in yield, loss in production of biomass and can also alleviate growth of plants (Verkleij et al., 2009). Toxicity of heavy metals can be studied in accordance with various factors. The first one is their oxidation state. It has been found that Cr (VI) has a higher toxicity than Cr (III) (Shanker et al., 2005). The soil pH can also affect the toxicity of a heavy metal. It has been observed that heavy metals are highly portable in acidic medium. They remain in the soil matrix in the forms where they are more bioavailable to the roots for absorption. A soil composition can give an advantage to heavy metal toxicity. Although there are certain plants called as hyper-accumulators which have a tendency to retain heavy metals and tolerate any harm caused by them. Generally such plants are a good choice for phytoremediation purpose (Watanabe et al., 1997).

Cadmium

The most popular heavy metal that is used in ceramic industries, television, electroplating processes, metallurgy, solders, insecticides, textile industries, photography and batteries is cadmium (Qui et al., 2018). Cadmium is one of the heavy elements that can enter into the food chain and cause some serious damage to biological systems. Tobacco smoking is the main entry path for cadmium into the human body. This may result into intense suffering of kidney and liver functions (Aoki et al., 2017) . Amongst aquatic animals Cd concentration were about 30 to 50 ppm in crabs and fishes which reside near industrial areas (Overnell et al., 1986). Cadmium is discharged through urine from the body but most of it is retained within causing problems in future (Talio et al., 2010).

Chromium

Chromium one of the profound elements has received special attention by environmentalists all over the world due to its high negative impacts on the ecosystem. Chromium generates mostly by anthropogenic activities such as tanning, mineral excavation, power plant cooling and energy production. Like any other heavy metal chromium does not degrade so it keeps accumulating in the environment and finally travels up to the food chain. Hexavalent chromium is considered to pose more danger to the environment than trivalent chromium (Mertz 1969; Saner 1980).

Source: ENVIS Centre, CPCB (www.cpcbenvis.nic.in)

Environmental Pollutant/Metalloids		Permissible limit (mg/l)
Lead	Pb	0.1
Cadmium	Cd	0.2
Zinc	Zn	5
Cyanides	CN	0.2
Nickel	Ni	2.0
Arsenic	As	0.2
Chromium hexavalent	Cr(VI)	0.1
Chromium (Total)	Cr	2.0
Manganese	Mn	2.0
Selenium	Se	0.05
Iron	Fe	3.0
Mercury	Hg	0.01
Vanadium	V	0.2
Fluorides	F	1.5
Chlorides	Cl	600
Copper	Cu	2.0
Boron	B	2.0
Phosphates	P	5.0
Sulphide	S	2.0
Sulphate	SO_4	1000
Ammonium nitrogen	NH_4	50

Table 1: Permissible limits of heavy metal pollutants

Impact of heavy metals on plants

Plants are sessile life forms and obtain their nutrition from soil itself. Soil consists of both essential and non-essential macro as well as micro-nutrients. They might be harmful after reaching a particular concentration (Xue et al., 2018). Plants have their own mechanism to excrete out excess metal but getting rid of a particular metal may lead to elevation in amount of some other metal which in turn proves harmful to the plant (Liang et al.,2018). The roots of plants have the anatomy that they can easily absorb even the slightest amount of required minerals for their metabolism. When industrial waste discharges huge amounts of metalloids, these plants are bound to absorb a huge amount of these metals as well. The first phase of damage occurs to the root cells as they directly come in contact with these metals (Rizvi and Khan, 2018). Steadily heavy metal toxicity starts affecting the homeostasis. Enzymes are also affected adversely which can hinder processes of metabolism. Photosynthesis, germination and growth are depleted leading to death of plant. Many side effects that include chlorosis, wilting, rolling of leaves and reduction of biomass generation also occur (Sanglam 2016; Shahid 2017).

Heavy metal	**Side effects on plants**	**Reference**
Copper (Cu)	• Oxidative stress • Production of reactive species	Filetti et al.,2018
Cadmium (Cd)	• Stunted growth • Acceptance of oxidative harm	Brendova et al.,2016
Zinc (Zn)	• Affected metabolic activity • Acceptance of oxidative harm	De Oliveira et al.,2018
Lead (Pb)	• Irregular morphology in plants	Kushwaha et al.,2018
Nickel (Ni)	• Nutrient imbalance • Scattering of cell membrane retention	Mendez et al.,2014
Chromium (Cr)	• Affects carbon dioxide fixation • Affects photophosphorylation and ETC	Mustafa et al.,2016
Arsenic (As)	• Leaf putrefaction • Root straining • Slows down shoot development	Kumari et al.,2018
Mercury (Hg)	• Physiological issues	Xun et al.,2017
Manganese (Mn)	• Chlorosis	Santos et al.,2017
Iron (Fe)	• Weakens cell structures • Harms membrane proteins and DNA	Mustafa et al.,2016

Table 2: Adverse effects of heavy metals on plants

Impact of heavy metals on human health

Exposure to heavy metals for a long term can cause significant damage to the central nervous system. They can bring harm to many vital organs like kidney, lungs, gastro-intestinal tract etc. As a matter of fact they can bring about alterations in blood composition. It has been mentioned in literatures that metalloid pollution is a cause of cancer, sclerosis and Alzheimer's disease. Heavy metals may enter into the body by ingesting, inhaling or via skin (El-Kady et al., 2018). Certain heavy metals are soluble in water and are ingested along with it. Other heavy metals like mercury enter into the body through our diet like fishes (Camacho et al., 2015). Mercury present in the atmosphere can be easily inhaled (Wang et al., 2004). A few metals like nickel can easily penetrate our skin (Tuchman et al., 2015).

Nanoremediation: An environment cleanup strategy

With the raging rate of industrialisation, the health of environment which sustains us is declining. It is an important issue to bring light upon. (Jadhav et al., 2013) reported high chloride concentrations with 0.75 ppm dissolved oxygen in aquifers. (Kale,2010) reported high amounts of heavy metals (Pb, Cd, Cr and Ni) in groundwater. The constant generation of pollutants (pesticides, grease, oil, dyes and heavy metals) brings devastation to our ecosystem and measures are required to stop it.

Nanotechnology has been anticipated to bring revolution in 21st century. A rapid growth in the field is observed all round the globe. One of the outcomes of research in the field of nanotechnology is the production of nanoparticles. These are particulates below the size of 100 nanometers. Some nanoparticles such as quantum dots are almost zero dimensions. Metallic nanoparticles are of great interest with respect to their electrical, optical, magnetic and catalytic properties (Bar et al.,2009). It is noted that nanoparticles have a tendency for detoxification of environmental pollutants. At the least they can transform pollutants into some neutral forms thus, minimising threats to ecosystem. Aquatic environments have been studied for the nature and behaviour of nanoparticles (Bundschuh et al., 2018). Nanoremediation can thus be considered a worthwhile strategy to cleaning harmful pollutants from environment (Rajan, 2011). Nanoremediation techniques prove to be inexpensive and require fewer infrastructures. The power consumption is less (Loffler and Edwards,2006). (Zhang, 2003) mentioned in a literature that iron nanoparticles have good surface area and a remarkable surface activity. Zero valent iron nanoparticles right now plays major role in environment clean up (Garner and Keller,2014).

Pollutants	Nano-remediation techniques	References
Chlorinated compounds	nZVI injection	Glazier et al.,2003
	Bimetallic particles	Yan et al.,2010
Pd, Cr, Cu	nZVI particles	Gheju et al.,2011
Hexachlorobenzene	Bimetallic particles	Nie et al.,2013
As(III), As(V)	Graphite nZVI composites	Wang et al.,2014a

Bisphenol	Hybrid nanoparticles	Wang et al.,2014b
Activated sludge	nZVI particles	Feng et al.,2014
Hg, Cd, As	Nanocomposites	Thatai et al.,2014
Pharmaceutical drugs	Carbon nanotubes	Xu and Bhattacharya, 2005
Zn^{2+}	nZVI particles	O'Carroll et al.,2013
Cr(VI)	Agarose stabilized (nZVI particles)	Jiao et al.,2014
Nitrates	Titanium nano memembranes	Bhattacharya et al.,2013
Explosive componds	Bimetallic particles	Koutsospyros et al.,2012

Table 3: Application of nanoparticles in environmental remediation

Green synthesis of nanoparticles

Advancement in technologies cannot be achieved at the cost of environment safety. In the same way there are many ways for synthesis of metallic nanoparticles but green synthesis is the one we opt here. The method approves of co-existence of environment and technology. Within biogenic synthesis there is a wide variety of methods to be explored. In the past nanoparticles have been synthesized from plants, fungi, bacteria etc. They have been called "bio-labs" for nanoparticle production.

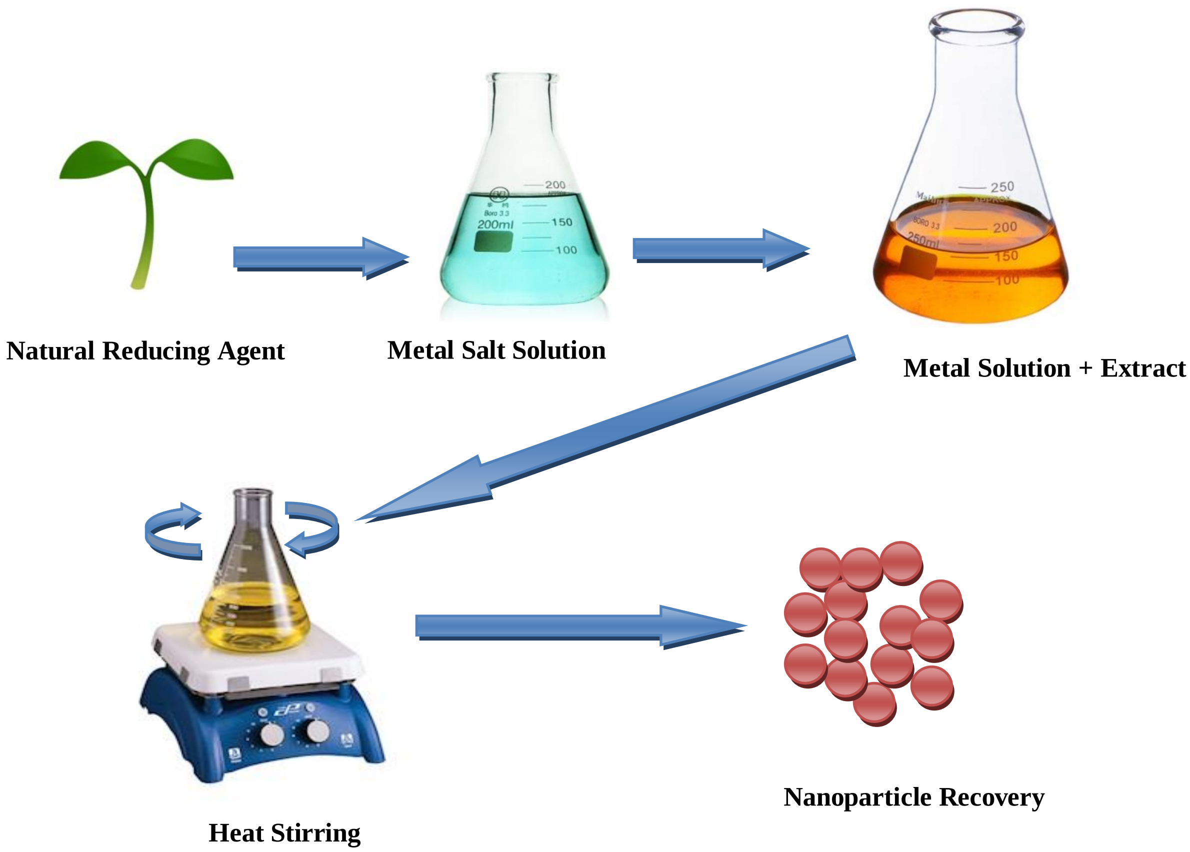


Figure 4: Schematic representation of biogenic synthesis of nanoparticles

Bacteria have the ability to reduce metal salts and thus forming metallic nanoparticles. The process includes both intracellular and extracellular cell functions. Organic functional group present in bacterial cell wall aid in reduction process. Many metallic nanoparticles have been synthesized using different strains of bacteria (Sunkar and Nachiyar 2012).

Bacteria	Nanoparticle synthesized	Reference
Bacillus cereus	Ag	Ganesh Babu and Gunasekaran, 2009
Pseudomonas aeruginosa	Au	Husseiny, 2007
Bacillus thuringiensis	Ag	Jain et al., 2010
Escherichia coli	Ag	Gurunathan et al.,2009
Escherichia coli --	Cds-	Sweeney et al., 2004
Marinobacter pelagius	Au	Sharma et al.,2012
Lactobacillus	Ag	Sintubin et al.,2009
Pseudomonas stutzeri	Ag	Klaus et al.,1999
Lactobacillus	Au	Sintubin et al.,2009
Desulfovibrio desulfuricans	Pd	Yong et al.,2002
Corynebacterium	Ag	Zhang et al.,2005
Acinetobacter sp. KCSI1	ZrO_2	Suriyaraj et al., 2019
Ureibacillus thermosphaericus	Ag	Juibari et al.,2011
Staphylococcus aureus	Ag	Nanda and Saravanan, 2009

Table 4: Biogenic synthesis of metallic nanoparticles from bacteria

Another yet effective route of synthesis of nanoparticles is by actinomycetes. It has been stated that it's an intracellular process. The metal ions are trapped in cell wall of mycelia of actinomycetes by carboxylate ions (electrostatic interaction) and are reduced to nanoparticles. The polydiversity of nanoparticles is good. They are also very stable and have great biocidal features towards pathogens.

Actinomycetes	Nanoparticle synthesized	Reference
Streptomyces hygroscopicus	Ag	Sadhasivam, 2010
Streptomyces sp.	Zn/Mn	Waghmare et al., 2011
*Streptomyces sp.*VITPK1	Ag	Sanjenbam et al., 2014
Streptomycetes viridogens	Au	Balagurunathan, 2011
Streptomyces albidoflavus	Ag	Prakasham et al., 2012
Thermomonospora sp.	Au	Sastry, 2003
Streptomyces sp.	Zn	Rajamanickam et al., 2012
Streptomyces hygroscopicus	Au	Waghmare et al., 2014
Streptomyces sp.	Zn/Cu	Usha, 2010
Nocardia farcinica	Au	Oza et al., 2012

Table 5: Biogenic synthesis of nanoparticles from actinomycetes

Very similar to bacteria, fungi also possess the ability to reduce and stabilize nanoparticles. They have enzymes and proteins which help them in the reduction process (Khandel and Shahi 2018). As mentioned in literature by Nayak et al., 2018 the yield of nanoparticles is better with fungi when compared to bacteria. They are easily handled by simplest of nutrients (Moghaddam et al., 2015). Some examples of these fungi are given below.

Fungi	Nanoparticle synthesized	Reference
Aspergillus sp.	Ag	Vigneshwaran et al., 2007
Fusarium sp.	Au	Philip, 2009
Penicillium sp.	Ag	Kathiresan, 2009
Trichothecium sp.	Au	Ahmad et al., 2005
Penicillium italicum	Ag	Nayak et al., 2018
Saccharomyces cerevisiae	Ag	Niknejad, 2019
Yeast strain MKY3	Ag	Kowshik et al., 2003
Klebsiella pneumoniae	Se	Fesharaki et al.,2010
Rhodospiridium dibovatum	PbS	Seshadri et al., 2011

Table 6: Biogenic synthesis of nanoparticles from fungi

The presence of electronic charge on algal cell wall promotes growth and nucleation. This provides rapid and economical route for the production of nanoparticles (Baker, 2013). Capping agents are present in algal extracts. Polysaccharides regulate the shape as well as size of NPs. Stability of nanoparticles is good facilitated by the presence of terpenoids and flavanoids in algal extracts. These nanoparticles are mostly used for medical purposes (Kannan et al., 2013).

Algae	Nanoparticle synthesized	Reference
Spirulina platensis	Au	Suganya et al., 2015
Phaeodactylum tricornutum	CdS	Scarano & Morelli, 2003
Stoechospermum marginatum	Au	Arockiya Aarthi Rajathi, 2012
Lemanea fluviatilis	Au	Sharma et al.,2014b
Prasiola crispa	Au	Sharmaet al., 2014a
Sargassum muticum	Au	Namvar, 2015
Ecklonia cava	Au	Venkatesan et al., 2014
Chlamydomonas reinhardtii	Ag	Barwal, 2011
Euglena gracilis	Ferrihydrite NPs	Brayner et al.,2012
Chlorella vulgaris	Au	Annamalai & Nallamuthu,2015
Chaetomorpha linum	Ag	Kannan, 2013
Caulerpa racemosa	Ag	Kathiraven etal., 2015
Sargassum wightii	Au	Singaravelu, 2007
Klebsormidium flaccidium	Au	Sicard et al., 2010

Table 7: Biogenic synthesis of nanoparticles from algae

Viruses, enzymes, peptides and other biological molecules are employed to produce nanoparticles. Some examples of this type of biosynthesis is cowpea mosaic virus (Douglas, 2002), tobacco mosaic virus (Shenton et al., 1999), M13 bacteriophage (Mao et al., 2003), DNA (Mahtab et al.,1995; Shaiu et al., 1993) and Cowpea chlorotic mottle virus (Douglas, 1998).

Apart from all the above mentioned routes the most common is using plants for production of metallic nanoparticles. This is due to the fact that the continent is rich in terms of herbs. Most of them are utilized in medicines. On the other hand our strong belief in Ayurveda and its influence in science cannot be denied. The process is rather free of complicated protocols. Scaling up the process is simple. A single plant provides abundant parts to be utilized in more than one experiment. The various parts used in nanoparticle synthesis as mentioned in previous literature are leaves, flowers, bark, fruits, roots and stem (Akhtar et al., 2013). Particle size of sixteen to twenty eight nanometeres has been concluded by Gopinath et al., 2012. Mubarakali et al., 2011 reported antimicrobial properties of nanoparticles synthesized by *Mentha piperita*. *Podophyllum hexandrum* synthesized AgNPs block cell functions of HeLa damaging DNA as reported by Jeyaraj et al., 2013. *Cissus quadrangularis* synthesized titatnium dioxide nanoparticles are also bacteriocidal reported by Priyadarshani et al., 2020.

Cinnamon has also been used used to generate AgNPs with antimicrobial activities (Premkumar et al., 2018). Antimitotic properties of ZnO NPs have also been observed. Cytotoxicity studies are done on HCT116 cell lines. AgNPs used were synthesized from *Caesalpinia pulcherrima*. In similar fashion zinc oxide and gold nanoparticles were generated from *Berberis aristata* leaves and leaf extract of *Mussaenda glabrata* respectively (Chandra, 2019; Francis, 2017). Antifungal properties of nanoparticles has been reported in literature by Jamdagni et al.,2018. Cytotoxicity studies on 4T1 and C26 cancer cells were done. FeNPs involved in the study were synthesized using rosemary. Some examples of plant synthesized nanoparticles are given in the table below.

NPs synthesized	**NP synthesized**	**NP synthesized**	**NP synthesized**
Au	*Aloe vera*	Leaves	Chandran, 2006
	Emblica of ficinalis	Fruit	Ankamwar, 2005
	Cyymbopogon flexuosus	Plant extract	Shankar et al.,2011
	Cicer arietinum	Bean extract	Ghule, 2006
	Beta vulgaris	Sugar beet pulp	Castro, 2011
	Nyctanthes arbortristis	Floral extract	Das, 2011
	Cuminum cyminum	Seed	Krishnamurthy, 2011
Ag	*Platanus orientalis*	Leaves	Song, 2009
	Ocimum sanctum	Root stem	Ahmad, 2010

	Tanacetum vulgare	Fruit	Dubey et, 2010
	Syzygium cumini	Seed	Kumar et al., 2010
	Sorghum spp	. Bran powder	Njagi,2010
	Curcuma longa	Tuber powder	Sathishkumar, 2010
	Shorea tumbuggaia	Stem bark	Savithramma, 2010
	Zingiber of ficinales	Rhizome	Kumar et al., 2012
Pd	*Gardenia jasminoides*	Leaves	Jia et al., 2009
	Pinus resinosa	Bark	Coccia et al., 2012
	Curcuma longa	Tuber	Sathishkumar et al.,2009
	Musa paradisica	Banana peel	Banker et al., 2010
Pt	*Doipyros kaki*	Leaves	Song et al., 2010
	Pinus resinosa	Bark	Coccia, 2012

Table 8: Biogenic synthesis of nanoparticles from plants

Mechanism of nanoparticle formation

For a long time plethora of studies have focused on various mechanisms by which nanoparticles are formed but none of the studies by far have established a basic agreeable mechanism. Some of the basic processes no matter remain constant in all of the studies.

Some of the hypothesis include the following. A study by NCL, Pune presented that presence of terpenoids have reduction effect on metal salts (Rautaray et al.,2005). Proteins on surface of gold nanoparticles were found (Shankar et al.,2003b). Reports suggest that tartaric acid in tamarind leaf extract acts as capping agent in gold nanoparticle formation (Ankamwar et al., 2005). The synthesis of AuNPs was reported by Gardea-Torresdey, 2002. The formation of spherical shaped AgNPs was explained in previous literature (Li et al.,2007). Huang et al., 2007 said that polyols are responsible for reduction of $AgNO_3$. Palladium nanoparticles were reduced from respective metal salt and were observed to be capped by caffeine. Coffee and tea extracts were used in the experiment (Nadagouda and Varma,2008). After analysing previous literature a sketch of the supposed mechanism was outlined. This includes four main steps i.e.; reduction of the metal salts such as $AgNO_3$, $CuSO_4.5H_2O$ etc, nucleation and growth of nanoparticles. Finally capping and stabilization occurs (Akhtar et al., 2013).

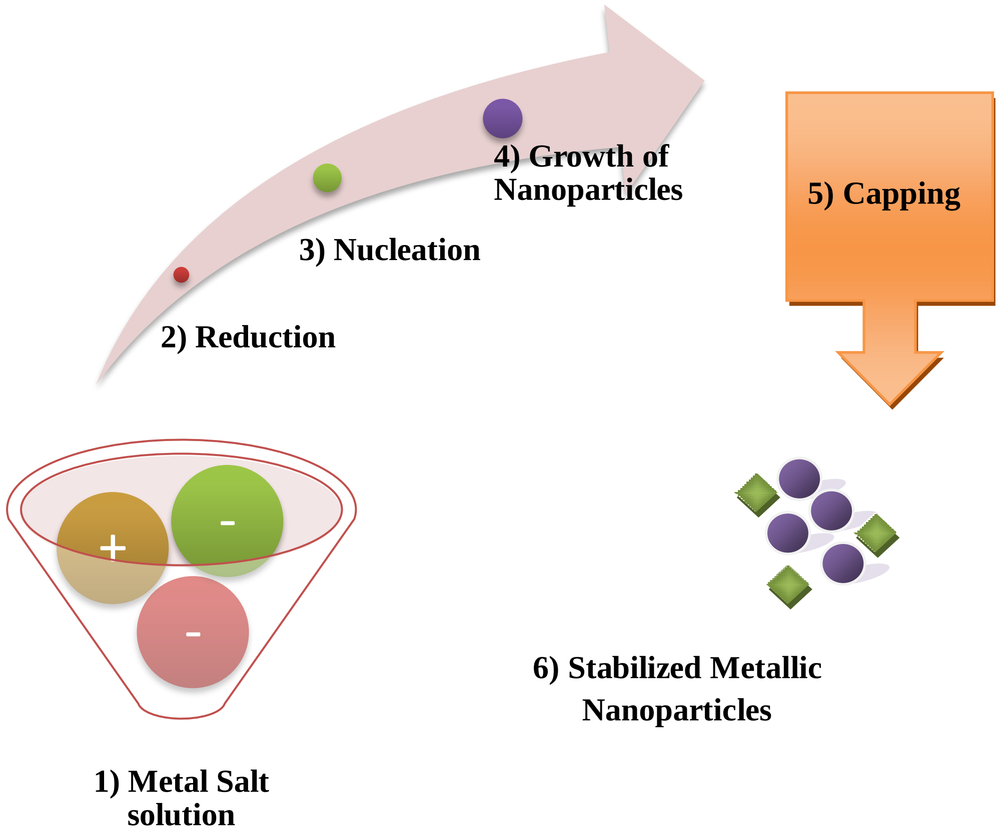


Figure 5: Steps involved in formation of nanoparticles

Applications of nanoparticles

There are countless applications of nanoparticles worldwide. Antimicrobial actvity being the most common of them all. As mentioned in literature AgNPs have been tested against both gram positive and negative bacteria. AgNPs has bacteriocidal properties against MDR bacteria (Rai et al.,2012; Morones, 2005). Nanoparticles are also used in biosensors (Hong et al.,2006). Nanoparticles aid in drug delivery systems stated by Pandey and Khuller, 2007. HIV interaction with NPs upto nanometer size has been studied by Elechiguerra et al.,2005. Cosmetics, toothpaste and other daily household commodities incorporate NPs to enhance their properties (Yu et al.,2011). In the field of agriculture fertilizers that release slowly are designed using NPs in order to prevent environment degradation (DeRosa,010). Apart from the above stated areas nanoparticles have enhanced crops (Torney et al.,2007), used for immobilization of proteins (Liu et al.,2003), function in bioremediation. They play important role in diagnosis and therapy (Gao et al.,2008). They are key elements in modern technology.

CHAPTER 3
MATERIAL AND METHODS

Plant material and chemicals

Catharanthus roseus Linn was identified within Delhi Technological University campus, Delhi. Fresh leaves were harvested. The specimen was labelled and stored further for referential purpose. Salts of silver nitrate and copper sulphate were obtained from Plant tissue culture laboratory DTU. Heavy metal salt for chromium and stock solution of cadmium was also obtained from the same laboratory. Nutrient broth (NB) media, Nutrient agar (NA) and streptomycin were purchased from Hi-media (Mumbai, India). All the chemicals were assured of high quality and purity.

Preparation of plant extract

C. roseus leaves were washed thoroughly and dried for about an hour. 10 grams of leaves were weighed and were added to a 200 ml beaker. 100 ml of milli-Q was added to the beaker. The beaker was heated to 60 ^{0}C for about 10 minutes to procure leaf extract. The beaker was allowed to cool off. Later the extract was filtered (using Whatman filter paper no. 1) into a 200 ml Erlenmeyer flask. Prepared plant extract was stored at 4 ^{0}C.

Preparation of metal salt solutions

Different concentrations (namely 1 mM, 3 mM and 5 mM) of silver nitrate and copper sulphate solutions were prepared by adding the calculated amounts of salt into 100 ml of milli-Q water. The flasks containing salt solutions were covered in aluminium foil and stored at room temperature.

Synthesis of nanoparticles

To synthesize silver nanoparticles 90 ml of 1 mM $AgNO_3$ salt solution was mixed with 10 ml of plant leaf extract. The solution was then kept in an incubater shaker at 45 ^{0}C at 100 rpm for 24 hours.

Similar procedure was performed to synthesize copper nanoparticles where 90 ml of 1 mM copper sulphate salt solution was mixed with 10 ml of plant leaf extract. The solution was then kept in an incubater shaker at 45 ^{0}C at 100 rpm for 24 hours.

The solutions were analysed using UV-Visible spectrophotometer (Perkin Elmer).

Optimization of nanoparticles

- ***Effect of salt concentration on NP synthesis***

  To determine the effect of concentration of salt solution on nanoparticle synthesis varied concentrations of $AgNO_3$ salt solution (1 mM, 3 mM and 5 mM) was mixed with plant leaf extract in the ratio 9:1. Similarily different concentrations of copper sulphate salt solution (1 mM, 3 mM and 5 mM) was added to plant leaf extract in the ratio 9:1. They were kept in an incubater shaker at 45 ^{0}C at 100 rpm for 24 hours. All six solutions were analysed using UV-Visible spectrophotometer (Perkin Elmer).

- ***Effect of plant extract concentration on NP synthesis***

  To determine the effect of concentration of plant extract, different volumes (1 ml, 2 ml and 3ml) plant extract was mixed with 1mM of silver nitrate salt solution. Similarily different volumes (1 ml, 2 ml and 3ml) plant extract was mixed with 1mM of copper sulphate salt solution .The solutions were kept in an incubater shaker at 45 ^{0}C at 100 rpm for 24 hours. All six solutions were analysed using UV-Visible spectrophotometer (Perkin Elmer).

- ***Effect of temperature on NP synthesis***

  To determine the effect of temperature on nanoparticle synthesis, 1:9 ratios of plant leaf extract and salt was mixed for both silver and copper in three different flasks. Each flask was incubated at 100 rpm for 24 hours at varied temperatures (25 ^{0}C 35 ^{0}C 45 ^{0}C). All six solutions were analysed using UV-Visible spectrophotometer (Perkin Elmer).

- ***Effect of time on NP synthesis***

  To determine how time affects nanoparticle synthesis, 1 mM of silver and copper salt solution was added to plant leaf extract in the ratio 9:1. Three different sets of nanopartcle solutions were prepared. One set was incubated at 45 ^{0}C at 100 rpm for 24 hours, while the other two sets were incubated in same condition for 48 hours and 72 hrs respectively. All six solutions were analysed using UV-Visible spectrophotometer (Perkin Elmer).

Characterization of nanoparticles

- ***UV-Vis spectroscopy***

  Ultra violet visible spectroscopy can be used to detect the formation of nanoparticles in a solution very conveniently. Periodic sampling of 1 ml aliquots of solution was done to monitor bio-reduction of silver and copper ions. UV-Visible spectra of solutions were measured as a function of time by Perkin Elmer UV-Visible spectrophotometer. Wavelength range for silver nanoparticle was 300-700 nm and the range of copper nanopaticles was 200-700 nm.

- ***Scanning electron microscope (SEM) analysis***

  Imaging of sample using SEM is done by scanning the sample with high energy beam of electrons. It uses a raster scanning pattern. SEM analysis was performed using Carl Zeiss EVO SEM machine at Amity University. Very small amounts of sample were dropped and thin films of nanoparticle samples were prepared on copper grid coated with carbon. Extra amount of sample was cleared with blotting paper. The film was dried under mercury lamp for 6-7 minutes.

- ***Fourier transform infrared (FT-IR) spectroscopy analysis***
  FT-IR analysis was done to determine organic groups that were involved in the bio-reduction process of silver and copper ions. Identification of capping agents was possible with the help of FT-IR. Both silver and copper nanoparticle solutions were centrifuged and sent for FT-IR analysis to Jamia Millia Islamia.

- ***Dynamic light scattering (DLS) analysis***
  The technique uses variation of time of scattered light from suspended particles which are under Brownian motion. This helps in obtaining the size variation of particles in liquid medium. Dilute suspensions of silver and copper nanoparticles were sent for DLS analysis to Jamia Millia Islamia.

- ***Zeta potential measurement***
  Since the surface charge determination of liquid nanopartice solutions is unsatisfactory electrical potential of particle is studied. The potential taken at the shear plane is called zeta potential. It helps in determining the surface morphology, stability and surface adsorption studies of suspended particles. AgNP and CuNP suspensions were sent for characterization.

Preparation heavy metal stock solutions

To prepare 90 ppm chromium stock solution, a calculated amount of Chromium chloride salt was added to 50 ml of double distilled water. Eventually the stock was diluted to obtain 70 ppm, 50 ppm, 30 ppm and 10 ppm chromium stocks.
For cadmium stock solution preparation 1000 ppm stock solution was procured from laboratory and diluted to 90 ppm, 70 ppm, 50 ppm, 30 ppm and 10 ppm stock solution. Absorbance of chromium and cadmium stock solutions was taken using UV-Visible spectrophotometer (Perkin Elmer). A standard curve was drawn for chromium and cadmium.

Heavy metal removal using nanoparticles

1 ml of biogenic silver nanoparticles from *Catharanthus* leaf extract was added to 50 ppm stock solution chromium and cadmium stock solutions. The absorbance of the mixture was taken at 0 hours. The mixture was then kept in an incubater shaker at 100 rpm at room temperature. Another reading for absorbance was taken after 24 hours.
1 ml of green synthesized copper nanoparticles from *Catharanthus* leaf extract was added to 50 ppm stock solution chromium and cadmium stock solutions. The absorbance of the mixture was taken at 0 hours. The mixture was then kept in an incubater shaker at 100 rpm at room temperature. Another reading for absorbance was taken after 24 hours. Percentage removal of heavy metals was calculated.

Preparation agar plates

Nutrient broth and nutrient agar media was prepared and autoclaved. The pH of media was adjusted to 7. Nutrient agar was poured into two petri-plates inside LAF chamber and left to solidify. 20 ml of nutrient broth was inoculated with *Staphylococcus aureus*. The inoculated media was given incubation at 37 ^{0}C overnight for the bacteria to grow.

Antibacterial testing

Antibacterial testing was performed using disc diffusion method. All the testing was done under sterile conditions inside LAF chamber to avoid any possible contamination. 100 µl of culture media was poured onto the agar plates and was spread evenly using a glass spreader. Four sterile discs were placed on the agar plant at proportioned distance in each plate.

In first plate one disc acts as control which is loaded with 30 µl of streptomycin. The other three discs were loaded with newly synthesized AgNPs. The amount of nanoparticle solution loaded was 10 µl, 30 µl and 50 µl respectively. Loading was done using micropipettes.

In second plate one disc acts as control which is loaded with 30 µl of streptomycin. The other three discs were loaded with newly synthesized CuNPs. The amount of nanoparticle solution loaded was 10 µl, 30 µl and 50 µl respectively. Loading was done using micropipettes. Both the plates were given incubation overnight at 37 ^{0}C. The plates were observed the next day.

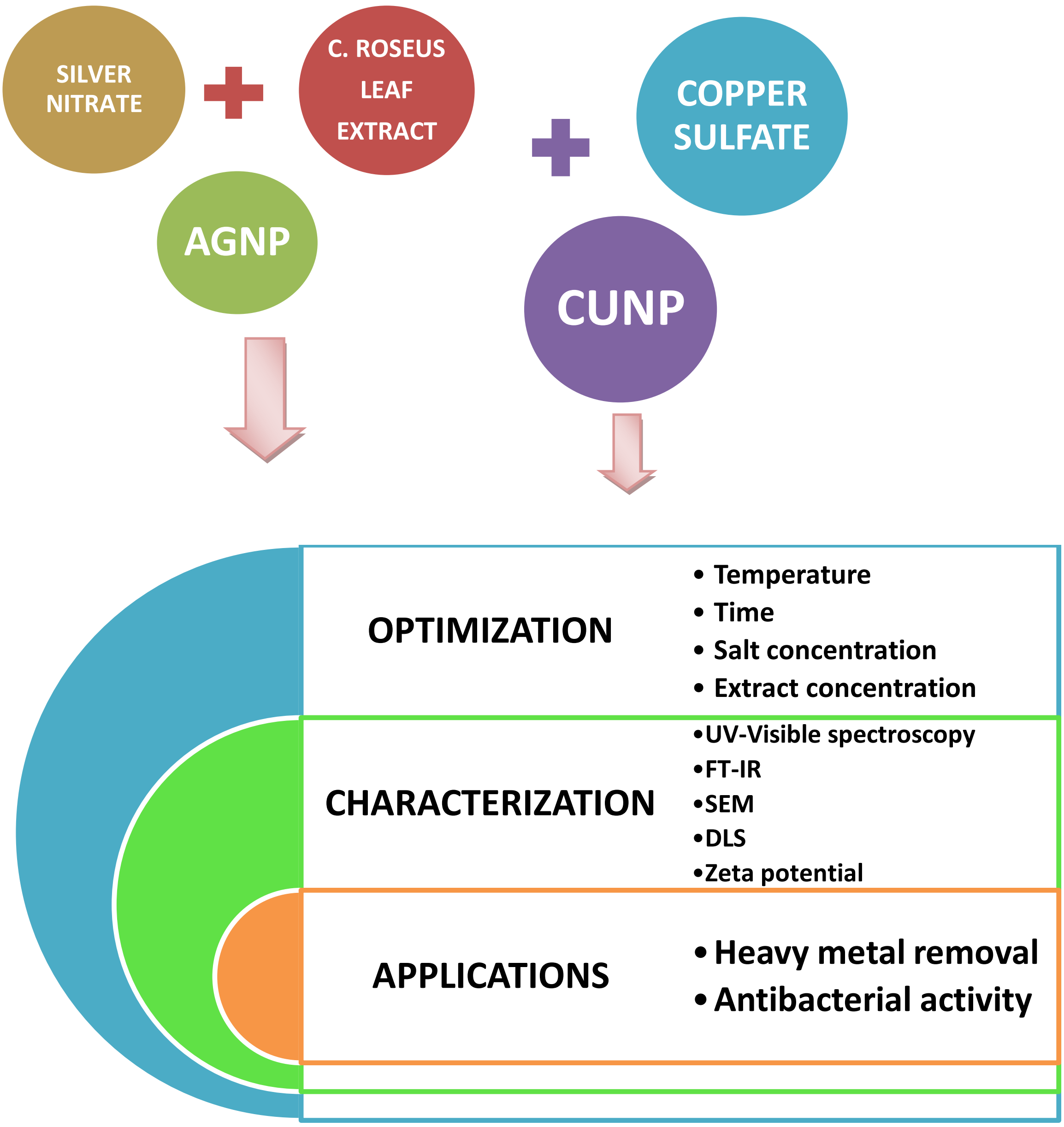


Figure 6: Schematic representation of synthesis of nanoparticles and their applications

CHAPTER 4
RESULTS AND DISCUSSIONS

1) Collection of plant material and Preparation of leaf extract

All the raw material for the plant extract collected from DTU campus shown in Fig. 7a) was utilized to prepare leaf extract shown in Fig. 7b). Prepared leaf extract was used for about one week and was stored at 4 ^{0}C.

Figure 7: Plant extract prepared from fresh *Catharanthus* leaves

2) Synthesis of silver and copper nanoparticles

Fig. 8a) shows silver nanoparticles were formed. This is indicated by change in colour intensity of silver nitrate solution from transparent to brown colour on addition of plant extract. Synthesis of copper nanoparticles is indicated by the change in colur intensity of copper sulphate solution from light blue to yellow in Fig. 8b)

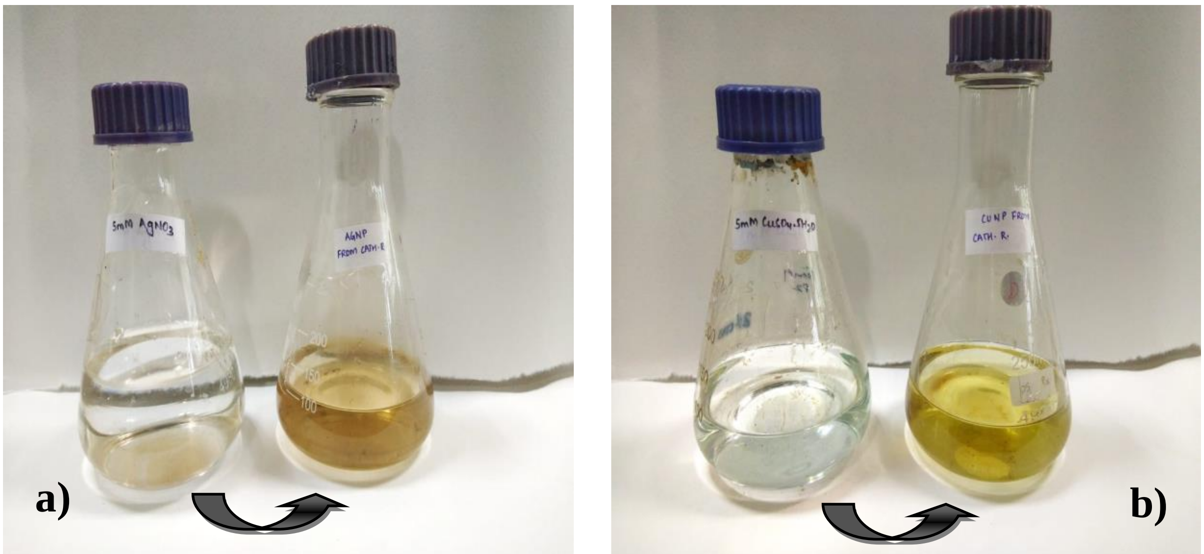


Figure 8: Silver and copper nanoparticles prepared from 5 mM $AgNO_3$ and CuNP

3) UV- Vis analysis and optimization of nanoparticles

Silver and copper nanoparticles were detected and optimized under various conditions metal salt concentration, leaf extract concentration, temperature and time.

- ***Effect of salt concentration on NP synthesis***

UV-Visible spectroscopy spectra prove that the higher the salt concentration, higher is the yield of nanoparticles. Both silver and copper nanoparticle yield was maximum at 5 mM concentration of silver nitrate and copper sulfate. Higher salt concentration provides more ions to be reduced and stabilized into nanoparticles.

- ***Effect of leaf extract concentration on NP synthesis***

Similarly, the higher the leaf extract concentration higher will be nanoparticle yield. In case of both silver and copper where 3 ml leaf extract was added to 7 ml salt solution, higher amount of nanoparticle formation was observed. This increases the amount of reducing agents in the process.

- ***Effect of temperature on NP synthesis***

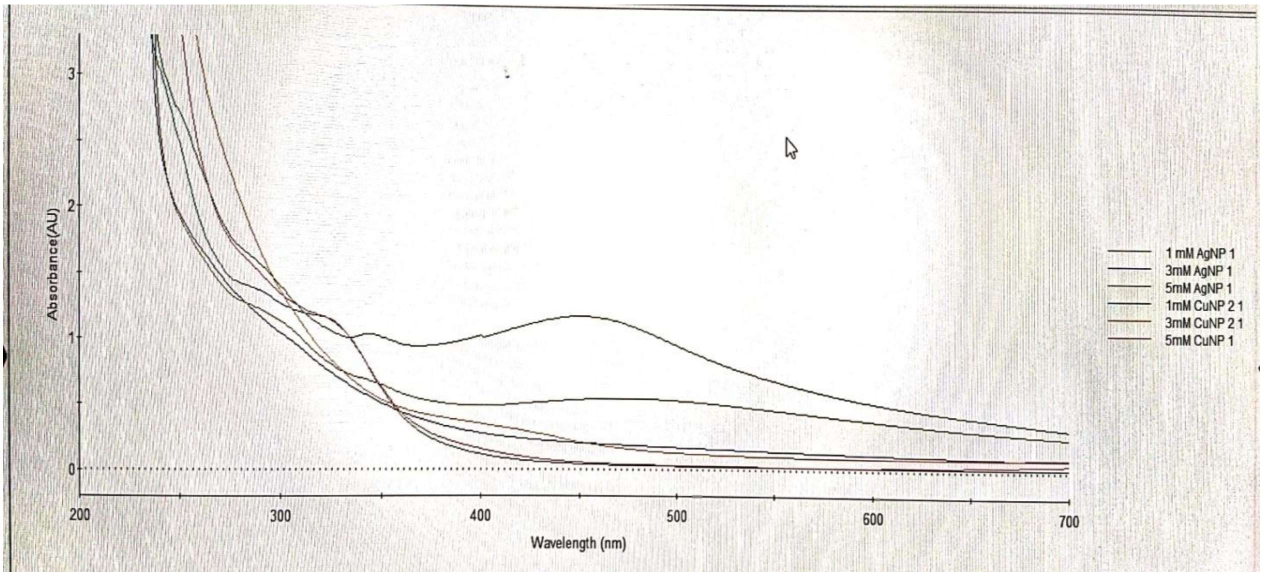
At 45 ^{0}C nanoparticle formation was maximum for both silver and copper. This indicates that higher temperatures can positively catalyse reduction process.

The effect of time is directly proportional to nanoparticle formation. This gives more reaction time which means reducing agents get more time to react with metal ions in order to form stable nanoparticles.

- ***Effect of time on NP synthesis***

Silver and copper NPs observed after 72 hours time period had more colour intensity. The above results were obtained by observing SPR peak of both NPs where maximum absorption takes place.

Absorption range for 300- 700 nm for AgNP and 200- 700 nm for CuNP.



Name	AU(200.000000000nm)	AU(210.000000000nm)	AU(220.000000000nm)	AU(230.000000000nm)	AU(240.000000000nm)	AU(250.000000000nm)	AU(260.000000000nm)	AU(270.000000000nm)	AU(280.
1 mM AgNP 1	4.4216	4.7114	4.8562	4.9609	3.1866	2.7148	2.297	1.9059	
3mM AgNP 1	4.4262	4.7278	4.8596	4.9415	2.5107	1.9382	1.6832	1.4755	
5mM AgNP 1	4.4155	4.7256	4.8663	4.9547	2.6354	1.9083	1.6327	1.412	
1mM CuNP 2 1	4.4304	4.7212	4.882	4.3531	3.0511	2.4665	1.8714	1.5546	
3mM CuNP 2 1	4.4307	4.7206	4.871	4.9609	4.9956	4.9192	3.1099	2.4767	
5mM CuNP 1	4.4045	4.7325	4.8636	4.9426	4.9895	3.5008	2.3666	1.8746	

Graph 1: UV-Vis spectra of different concentrations of metallic nanoparticles

4) Scanning electron microscope (SEM) analysis

SEM provided details of morphology of synthesized silver and copper nanoparticles. SEM images were taken at different resolutions ie; 200 nm, 300 nm and 1µm. The synthesized nanoparticles were relatively spherical and the size lied between 1- 100 nm for most of the particles. Images for silver and copper nanoparticles are given below in Fig. 9-12.

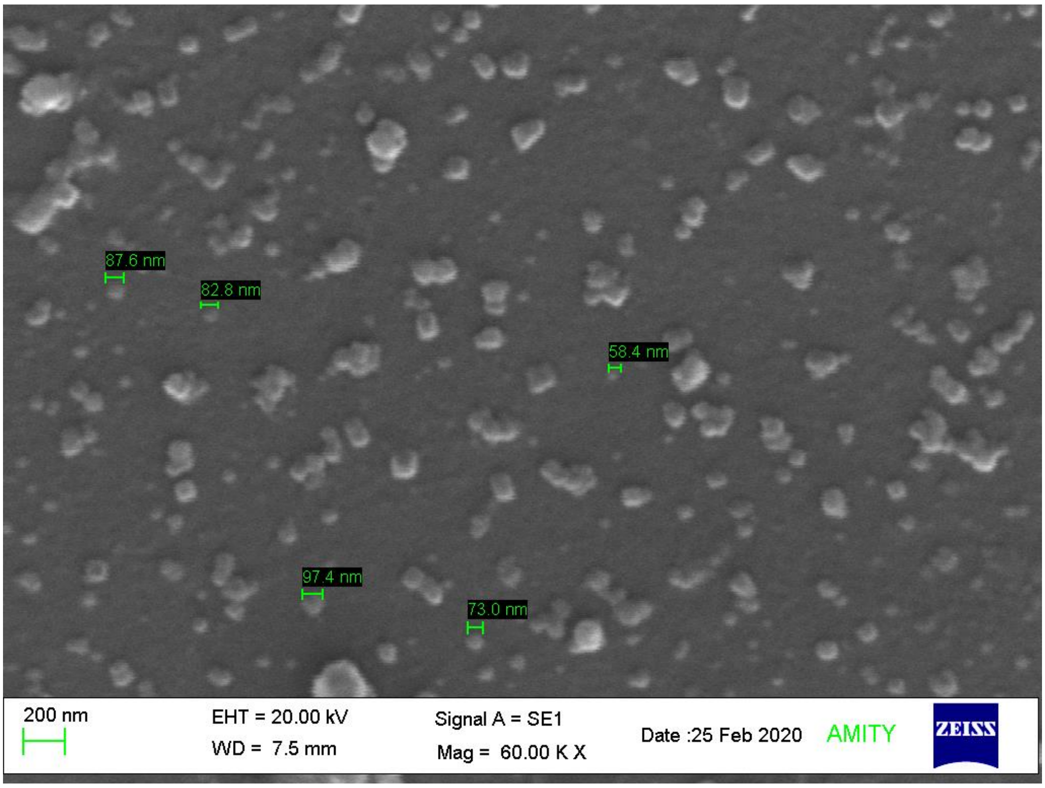


Figure 9: SEM image of silver nanoparticles at 200 nm resolution

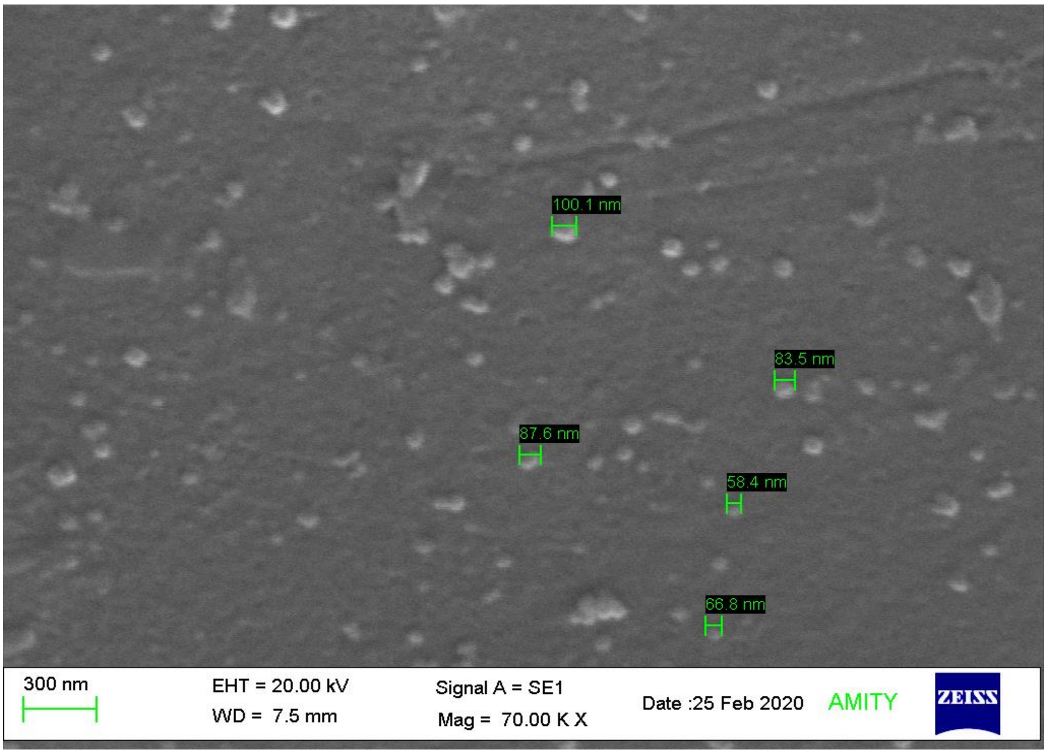


Figure 10: SEM image of silver nanoparticles at 300 nm resolution

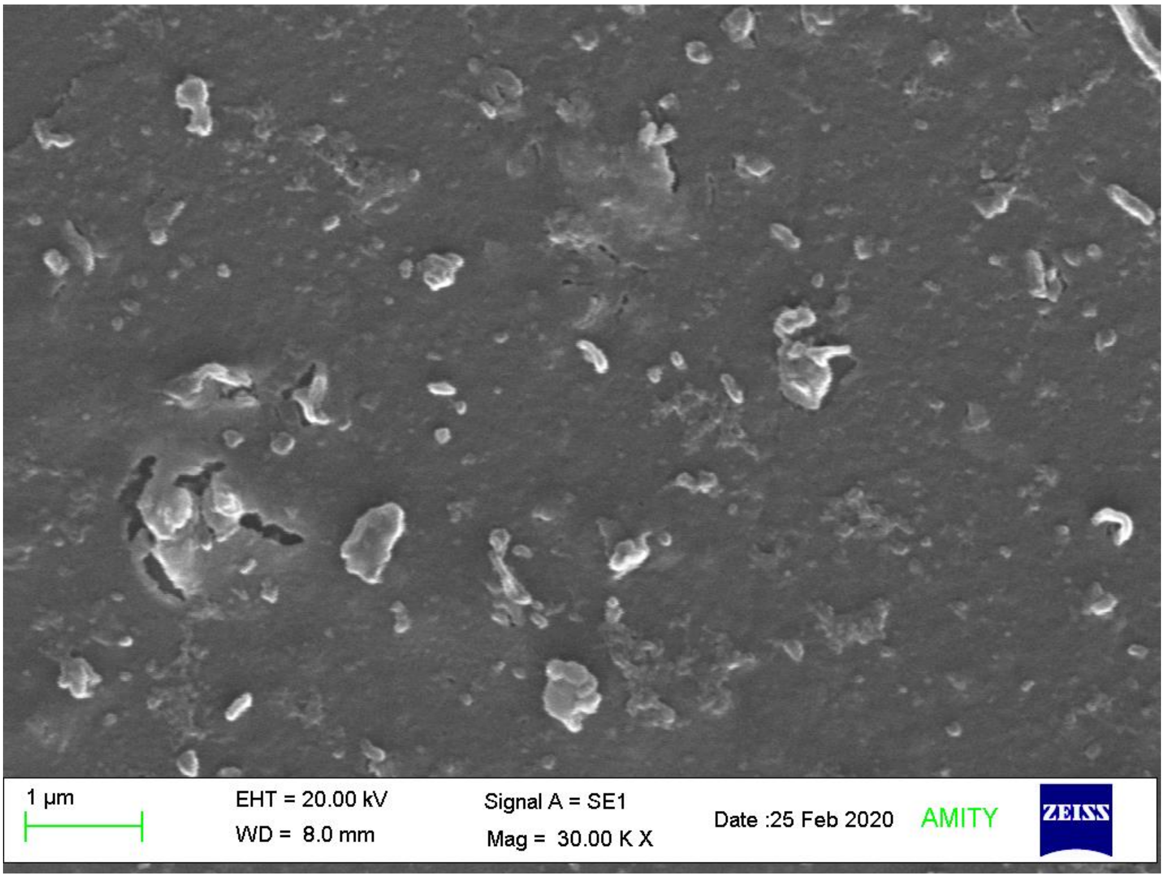


Figure 11: SEM image of copper nanoparticles at 1 µm resolution

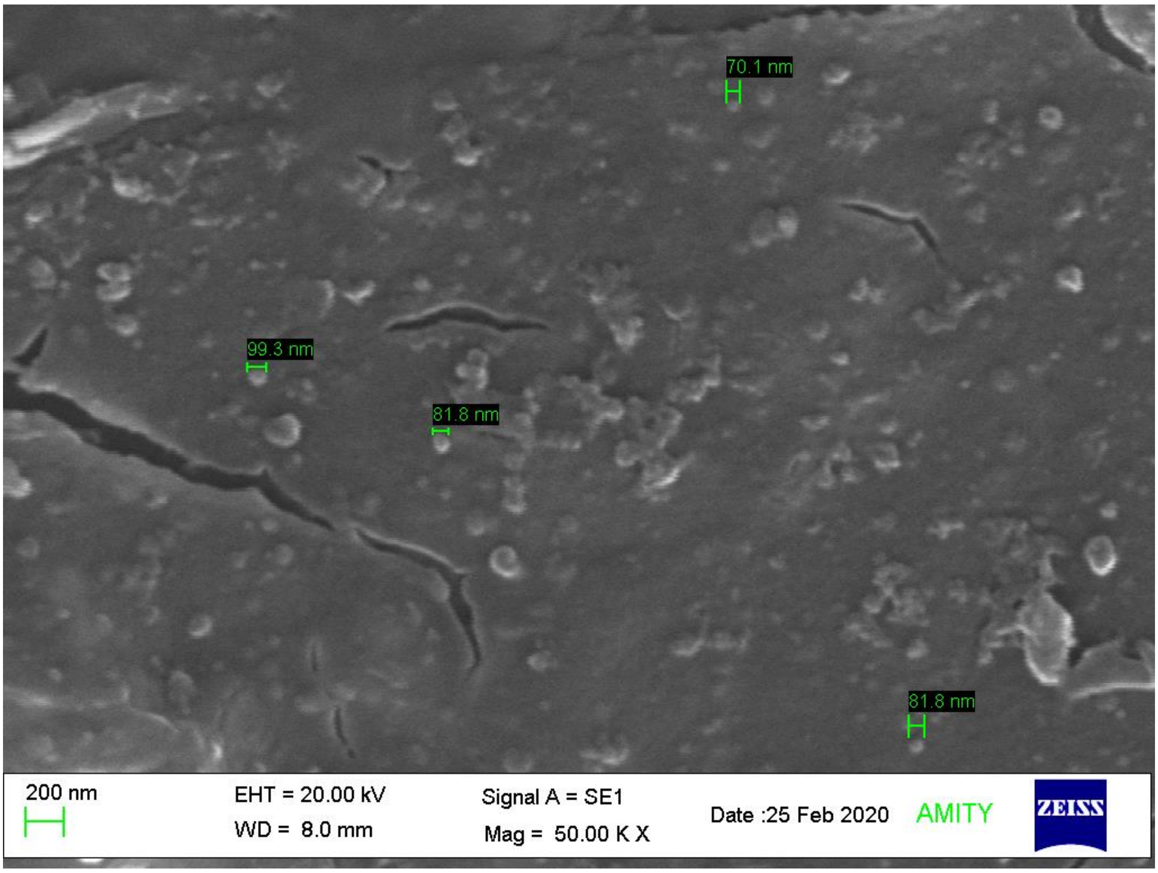


Figure 12: SEM image of copper nanoparticles at 200 nm resolution

5) Heavy metal removal

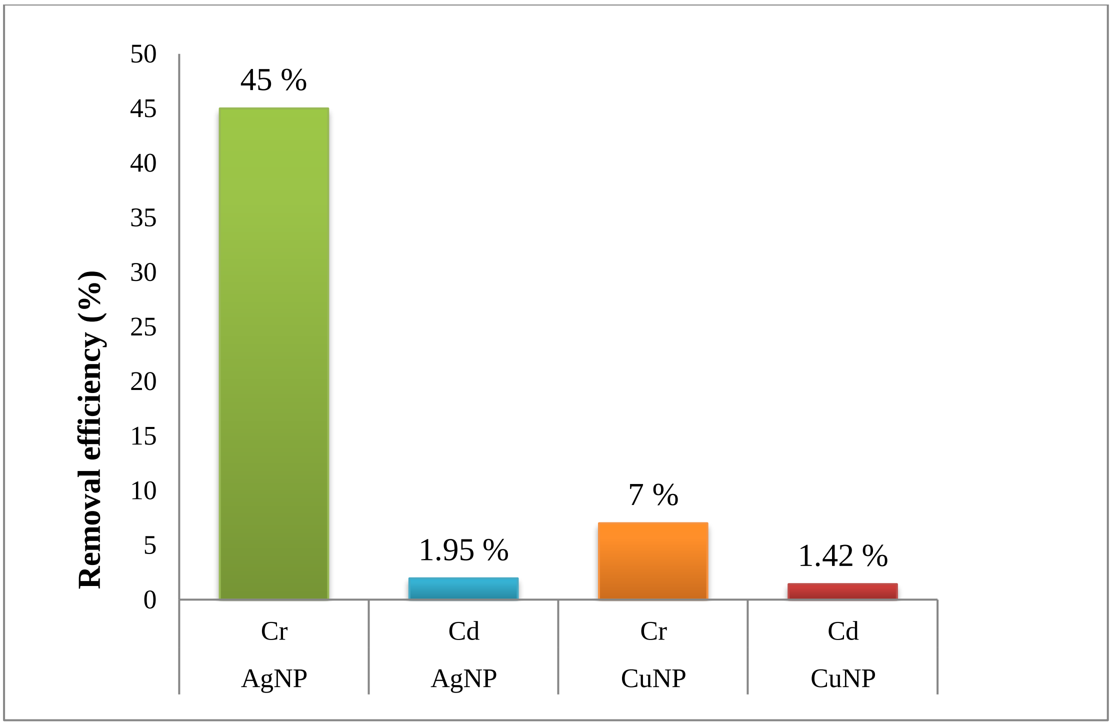
50 ppm of chromium and cadmium stock solutions were added with copper and silver nanoparticle solution respectively. Each aliquot were analysed using UV-Visible spectrophotometer. Wavelength used for chromium was 350 nm while wavelength used for cadmium was 300 nm. Absorbance was recorded at 0 hours after addition of nanopartcles. The second absorbance reading was recorded after 24 hours of addition of nanopartcle. Concentration of heavy metal salt corresponding to their absorbance at 0 hours and 24 hrs were calculated using standard curve. Heavy metal removal efficiency was calculated using the equation given below.

$$\boldsymbol{R}\% = \frac{\boldsymbol{C_0} - \boldsymbol{C_t}}{\boldsymbol{C_0}} \times \mathbf{100}$$

where, R% is the removal effiency of heavy metals. C_0 and C_t are concentration at 0 hours and 24 hours after addition of nanoparticles.

Heavy metal	Nanoparticle used	Concentration Used (ppm)	Wavelength used (nm)	Initial OD @0 hours	Final OD @24 hours	Removal efficiency (%)
Cr	AgNP	50	350	0.3059	0.4436	45
Cd	AgNP	50	300	0.2146	0.2341	1.95
Cr	CuNP	50	350	0.3714	0.3975	7
Cd	CuNP	50	300	0.2217	0.2359	1.42

Table 9: Removal efficiency of green synthesized silver and copper nanoparticles



Graph 2: Cd and Cr removal efficiency by AgNP and CuNP

6) Antibacterial Testing

Biosynthesized copper and silver nanoparticles were studied for their bacteriocidal properties using standard microbiology assay of zone of inhibition (ZOI). For the experiment disc diffusion technique was used. Antibacterial testing was done against gram positive bacterial *Staphylococcus aureus*. Control drug streptomycin of 10 mg/ml concentration was used. Different concentrations of silver and copper nanopartcles were used. Fig. 13 indicates that silver nanoparticles have a better antibacterial activity than copper nanoparticles.

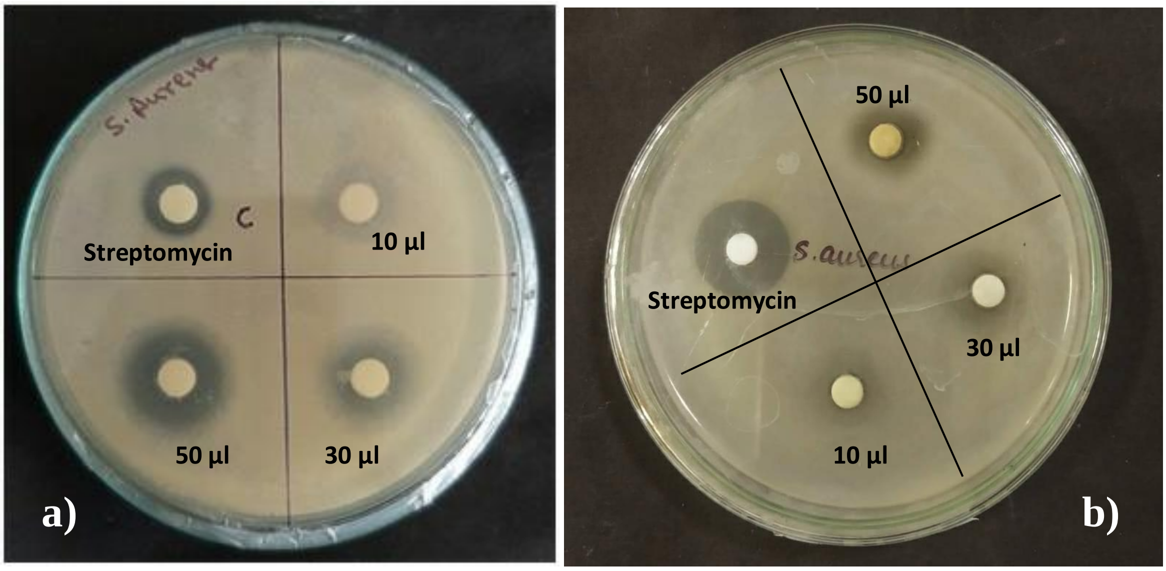


Figure 13: a) Effect of AgNP on *S. aureus* b) Effect of CuNP on *S. aureus*

Antibacterial activity of AgNP and CuNP synthesized from *C. roseus* is given below in Table 10.

Microbe	Nanoparticle	Control (Streptomycin) ZOI (mm)	Zone of Inhibition (mm)		
			10 µl	30 µl	50 µl
Staphylococcus aureus	AgNP	11	7	14	16
	CuNP	13	5	8	10

Table 10: Antibacterial testing using Zone of inhibition analysis

CHAPTER 5

CONCLUSION

Biogenic synthesis, moreover synthesis of desired nanoparticles from plants has numerous advantages over conventional methods with regard to the environment. The diverse range of raw materials available and simple protocols are an added benefit to the process. Many studies propose the synthesis mechanism of nanoparticles, most of them reveal the reduction metals into nascent ions. Citric acid, terpenoids, phenolics, hydrogenase etc constituted in plant extracts are engaged in the reduction process. These nascent ions are finally stabilized by capping agents resulting into nanoparticles. Most of the capping agents are functional groups like alcohols, ketones, aldehydes etc. Generally the range of nanoparticles formed is 1-100 nm but sometimes the particle size is increased due to protein binding. The morphology and number of nanoparticles synthesized are affected by various conditions. These conditions are pH, temperature, concentration of metal salts, concentration of plant extract and time.

The yield of silver nanoparticle was better than copper nanoparticles even when similar conditions were provided in both experimental setups. The nanoparticles synthesized in this study were both found to be spherical and within 100 nm range. The heavy metal removal capability of silver was found to be far superior than copper in case of chromium. Copper and silver have fewer tendencies to remove cadmium. This possibly could be the effect of reaction time and pH (Vaseghi et al., 2019). Silver and copper nanoparticles both have bacteriocidal effect against *S. aureus*. AgNPs results reveal a larger ZOI than CuNPs. Apparently the synthesis of both nanoparticles cost the same therefore, this establishes silver nanoparticles superior than copper nanoparticles.

FUTURE PROSPECTS

With a facile and economical technique for the production of nanoparticles, nano-biotechnology has a very promising future. Being environmentally benign it has been employed into various remediation processes. The biomedical applications are countless. They are currently used in biosensors, diagnosis and therapies. Nanoparticles possess antiviral, bacteriocidal and antifungal properties. Apart from fighting infections nanoparticles synthesized from plants can be used in cancer treatment, fighting infections and suppressing tumours. Wide spectrum of opportunities waits for the utilization of nanoparticles in different sectors, such as agriculture, bioremediation, nutraceuticals, cosmetics, polymers, food and beverages. Although much research has been done in the field still a larger area of nanotechnology remains unexplored.

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