



Plant-Mediated Synthesis and Characterization of Silver and Copper Oxide Nanoparticles: Antibacterial and Heavy Metal Removal Activity

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Abstract

The advancement in conservationist strategies for development of nanoparticles is elemental to the subject of nanotechnology. Green protocols are highly preferred over conventional methods as they are environmentally benign. Certain phytochemicals in plant extracts exhibit natural tendencies of bio-reduction of salts. They also possess the ability of stabilizing these reduced particles by capping them. In present study leaf extract of *Catharanthus roseus*, an evergreen subshrub has been utilized for production of AgNPs and CuO-NPs. Synthesized nanoparticles were evaluated for their cadmium, chromium removal and antibacterial potential against *S. aureus*. AgNPs and CuO-NPs were optimized by varying salt concentration, leaf extract concentration and time interval to obtain better yield. UV–Vis spectroscopy was used to detect biogenic AgNPs and CuO-NPs. Wavelength range used for AgNPs and CuO-NPs was 300–700 and 200–700 nm successively. The morphology of nanoparticles was determined to be spherical and within 100 nm using SEM images. FT-IR investigation confirmed the presence of amines and alcohols in AgNPs. IR spectra of CuO-NPs revealed the ubiquity of aldehydes/ ketones and carboxylic acids. The average distribution for silver was 602.9 nm and for copper was 1066 nm as confirmed by DLS analysis. Further zeta-potential for AgNPs and CuO-NPs was recorded -16.4 mV and -6.18 mV. Kirby Bauer test for *S. aureus* show maximum ZOI i.e. 16 mm in case of AgNP (50 µl) and 10 mm in case of CuO-NP (50 µl). Highest chromium and cadmium removal was observed in case of biogenic silver nanoparticles i.e. 47.84% and 5.68% respectively. This is the first work that presents a comparative study of biogenic AgNPs and CuO-NPs from the leaf extract of *Catharanthus roseus*. Our findings can also help in improving the current scenario of metalloids pollution in soil and water environments. Hence, proper scaling up can make biogenic AgNPs and CuO-NPs a noteworthy tool in industries as well.

Keywords *Catharanthus roseus* · Silver nanoparticles · Copper oxide nanoparticles · Biogenic synthesis · Cadmium removal · Chromium removal · Antibacterial

Introduction

Nanotechnology has been anticipated to bring revolution in twenty-first century. A swift rise in the area is observed all round the globe. One of the outcomes of research in nanotechnology is the production of nano sized particles. These are particulates below the size of 100 nm. Some nanoparticles such as quantum dots are almost zero dimensions. Metallic nano sized particles are of extreme

interest with respect to electrical, optical and catalytic properties [1]. It is noted that nanoparticles have a tendency for detoxification of environmental pollutants [2]. 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 [3]. Nanoremediation can thus be considered a worthwhile strategy to cleaning harmful pollutants from environment. Nanoremediation techniques prove to be inexpensive and require fewer infrastructures. The power consumption is less [4].

Advancement in technologies cannot be achieved at the cost of environment safety. There are a number of ways for the synthesis of metallic nanoparticles but green synthesis

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is considered best. Biological methods also called as biogenic/ biomimetic route for synthesis, is one of the most popular method of generating metal nanoparticles. 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 and chemical requirements are almost negligible when compared to sophisticated techniques.

Within biogenic synthesis there is a wide variety of methods to be explored. In the past nanoparticles have been synthesized from plants, fungi and bacteria, also called biolabs. 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 [5]. 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. Similarly, fungi also possess the ability to reduce and stabilize nanoparticles. They have enzymes and proteins which help them in the reduction process [6]. Reza synthesized zirconium nanoparticles from *Penicillium* species [7]. As mentioned in literature by Nayak et al., [8] amount of nanoparticles produced using fungi is more, when compared to bacteria. They are easily handled by simplest of nutrients [9]. In case of algae, the presence of electronic charge on cell wall promotes growth and nucleation. This provides rapid and economical route for generation of nano sized particles [10]. 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 [11]. Viruses, enzymes, peptides and other biological molecules are also employed to produce nanoparticles.

Apart from all the above mentioned routes the most common is using plants for producing metallic nanoparticles [12]. A vast diversity of plant having medicinal importance exists on earth. The green synthesis process is rather free of complicated protocols. Scaling up the process is simple. Various parts used in nanoparticle synthesis as mentioned in literature are leaves, flowers, bark, fruits, roots and stem [13]. Particle range of sixteen to twenty eight nanometres has been reported previously [14]. Mubarakali et al., 2011 [15] reported antimicrobial properties of biogenic NPs synthesized by *Mentha piperita*. *Podophyllum hexandrum* synthesized AgNPs block cell

functions of HeLa damaging DNA as reported by Jeyaraj et al., 2013 [16]. *Cissus quadrangularis* synthesized titanium dioxide nanoparticles are also bacteriocidal as reported by Priyadarshani et al. [17]. Cinnamon has also been used to generate AgNPs with antimicrobial activities [18]. AgNPs have been tested effective for gram positive as well as gram negative bacteria. They have bacteriocidal properties against MDR bacteria [19, 20]. Raina et al. [21] stated that silver and copper oxide nanoparticles are highly effective in catalytic and photocatalytic degeneration of various dyes like MO, PR, MR and EY. Nanoparticles are employed in biosensors [22]. Introduction of nanotechnology in field of medicine and imaging is highly anticipated. Nanoparticles aid in drug delivery systems stated by Pandey and Khuller [23]. HIV interaction with NPs upto nanometer size has been studied by Elechiguerra et al. [24]. Barabadi [25] concluded that antineoplastic gold and silver nanoparticles are effective against cervical, breast and prostate cancer cell line. AuNPs were also fabricated from aqueous extract of jellyfish and *Pongamia pinnata* for harvesting its anticancer potential [26]. Although a vast number of studies indicate efficiency of P-NPs in biomedical applications, there have been evidences of genotoxicity caused due to these nanoparticles. Genetic toxicity of a biosynthesized metallic nanoparticle is dependent upon its synthesis parameters, dose, time and specific concentration. Disregarding the side effects can pose a serious threat to human life therefore FDA recommends all drugs to be tested for any carcinogenic, mutagenic and teratogenic effects before drug trials and marketing such nanoparticle infused treatments. [27]. Cosmetics, toothpaste and other daily household commodities also incorporate M-NPs to enhance their properties [28]. In the field of agriculture fertilizers that release slowly are designed using NPs in order to prevent environment degradation [29]. Apart from the above stated areas nanoparticles have enhanced crops [30], used for immobilization of proteins [31], function in bioremediation. They play important role in diagnosis and therapy [32]. They are key elements in modern technology. Nanotechnology has made its way to solving the problem of metalloids pollution. It has been proven that carbon nanotubes can be used to remove Ar(V) ions from water [33]. In an investigation by Ali et al. [34] cellulose functionalized with Zn^{2+} and Ag^+ was capable of removing metal ions from water.

Increasing urbanization has resulted in the release of toxic pollutants like pesticides, heavy metals, dyes etc. in water bodies and soil [35]. Heavy metals are metallic elements that have normally higher atomic density than 4000 kg/m^3 . Toxicity of heavy metals is inevitable even if they are present in traces. Many of the heavy metals like Cu, Fe, B, Ni, Mo 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 [36]. Heavy metal exposure for a long period of time can cause significant damage to brain, spinal cord and other vital organs. Metalloid pollution is a cause of cancer, sclerosis and Alzheimer's disease [37]. Metallic nanoparticles illustrate widespread applications in the area of medicine and environmental remediation.

With a wide variety of methods available for synthesis of nanoparticles, it is important to select an eco-friendly, cost effective and simple methodology. Therefore, present study focuses on production of metallic nanoparticles from *Catharanthus roseus*. Besides being extensively available, the plant also has 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 [38]. The extract from leaves in ethanol has antidiabetic effects. This has been determined from the hypoglycaemic properties of its alkaloids [39]. *Catharanthus roseus* also possesses antimicrobial properties [40]. It shows 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 [41].

In the present investigation silver and copper oxide nanoparticles were prepared from leaf extract of *Catharanthus roseus*, a plant with high medicinal value. To obtain maximum yield of nanoparticles various parameters were standardized and synthesised nanoparticles were tested for cadmium and chromium remediation. A comparative antibacterial response of fabricated nanoparticles with reference to their size, morphology, yield, bactericidal property was also studied. In the last decade, countless studies have been conducted to produce plant mediated nanoparticles and testing their antibacterial potential. Finally, in this investigation we have focused biofabricating two nanoparticles in order to evaluate their potential against bacteria and also their contribution in environment remediation. The current study focuses on heavy metal removal in concentrated cadmium and chromium solutions. This can be expanded further for treatment of industrial wastewater sample. Optimization strategies will open opportunities for scaling up the process.

Materials and Methods

Catharanthus roseus Linn was identified within Delhi Technological University campus, Delhi. Fresh leaves were harvested. Salts of silver nitrate and copper sulphate were obtained from Plant tissue culture laboratory DTU. Stocks of heavy metals was prepared and kept for further use.

Nutrient broth (NB) media, Nutrient agar (NA) and streptomycin were purchased from Hi-media.

Preparation of Leaf Extract and Metal Solutions

C. roseus leaves were washed thoroughly and dried for about an hour. 10 g 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 °C for about 10 min 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 °C. 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 [42].

Synthesis of Nanoparticles

To synthesize AgNPs 90 ml of 1 mM AgNO₃ salt solution was mixed with 10 ml of *Catharanthus* leaf extract. The solution was then kept in an incubator shaker at 45 °C at 100 rpm for 24 h. Similar procedure was performed to synthesize copper oxide nanoparticles where 90 ml of 1 mM CuSO₄ salt solution was mixed with 10 ml of *Catharanthus* leaf extract. The solution was then kept in an incubator shaker at 45 °C at 100 rpm for 24 h.

Optimization of Nanoparticles

To determine the effect of concentration of salt solution on nanoparticle synthesis varied concentrations of AgNO₃ salt solution (1 mM, 3 mM and 5 mM) was mixed with plant leaf extract in the ratio 9:1 [43]. They were kept in an incubator shaker at 45 °C at 100 rpm for 24 h. To determine concentration effects of plant extract, different volumes (1 ml, 2 ml and 3 ml) plant extract was added with 1 mM of AgNO₃ solution. The solutions were kept in an incubator shaker at 45 °C at 100 rpm for 24 h. To determine how time affects nanoparticle synthesis, 1 mM of silver salt solution was added to plant leaf extract in the ratio 9:1. Three different sets of nanoparticle solutions were prepared. One solution was incubated at 45 °C at 100 rpm for 24 h, while the other two were incubated in same condition for 48 h and 72 h respectively. Similar procedure was repeated for copper oxide nanoparticles. All solutions were analysed using UV-Visible spectrophotometer (Perkin Elmer) for detecting formation of nanoparticles.

Heavy Metal Removal Using Nanoparticles

1 ml each of biogenic silver and copper oxide nanoparticles from *Catharanthus* leaf extract was added to 50 ppm chromium and cadmium stock solutions. The absorbance of the mixture was taken at 0 h. The mixture was then kept in an incubator shaker at 100 rpm at room temperature. Another reading for absorbance was taken after 24 h. Unknown concentration of heavy metal salt corresponding to their absorbance at 0 h and 24 h were calculated using standard curve [44]. Heavy metal removal efficiency was calculated using the equation given below.

$$R\% = \left[\frac{C_0 - C_t}{C_0} \right] \times 100$$

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

Antibacterial Testing

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 °C overnight for the bacteria to grow. Antibacterial testing were performed using disc diffusion method. 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 plate 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. Similarly another plate was prepared for CuO-NPs. Both the plates were given incubation overnight at 37 °C. The plates were observed the next day.

The complete summary of materials and methodology used is given in Fig. 1.

Results and Discussion

Visible Observation

Formation of AgNPs and CuO-NPs from leaf extract of *Catharanthus* is indicated by a visible colour change in both solutions. The phytochemicals existing in plant extract reduce silver nitrate and copper sulphate solutions. Extracellular synthesis is preferred for nanoparticle synthesis as it introduces lesser chemical impurities and makes

nanoparticle extraction simpler [45]. 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. After analysing previous literature a sketch of the supposed mechanism was outlined (Fig. 2). This includes four main steps i.e.; reduction of the metal salts such as AgNO₃, CuSO₄.5H₂O etc., nucleation and growth of nanoparticles. Finally capping and stabilization occurs [13]. Silver nitrate solution turns from transparent to dark brown (Fig. 3a) while copper solution turns yellow from a light blue solution (Fig. 3b). The colour changes occur because of deviation in surface plasmon resonance. Maximum colour change is observed in solution containing 3 ml leaf extract, 5 mm of salt solution and 72 h incubation. This is due to increase in number of ions and reaction time. Similar observations were made by Roy [42] and Raina [21] in their investigation.

UV-Vis Spectroscopy Analysis

Ultra violet visible spectroscopy was utilized to detect the formation of nanoparticles in a solution very conveniently. Bioactivity of nanoparticles is dependent upon several factors such as charge present on surface, functional group attached, morphology, structure and size of a nanoparticle. Different experimental attributes like temperature, pH, and concentration of reactants often impact surface chemistry of an inorganic nanoparticle thus influencing its biological activity [46, 47, 48, 49, and 50]. Silver and copper oxide nanoparticles were therefore optimized under various conditions like varying metal salt concentration [51], leaf extract concentration, [52] and time [53]. UV-Vis spectroscopy spectra prove that 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 sulphate. (Fig. 4) Higher salt concentration provides more ions to be reduced and stabilized into nanoparticles. Similarly, higher the leaf extract concentration higher will be nanoparticle yield. In case of both silver and copper where leaf extract and salt solution were added in the ratio 3:7, higher amount of nanoparticle formation was observed. (Fig. 5) This increases the amount of reducing agents in the process. Silver and copper NPs observed after 72 h time period had more colour intensity due to increase in reaction time. (Fig. 6) The above results were obtained by observing SPR peak of both NPs where maximum absorption takes place. The results are in agreement with previous literature [43]. Absorption range of 300- 700 nm was used for AgNP and 200- 700 nm was used for CuO-NP. Centrifugation of obtained AgNP and CuO-NP solution was done and it was found that silver nanoparticle solution gave higher amount of precipitate

Fig. 1 Schematic representation of materials and methodology used in current study

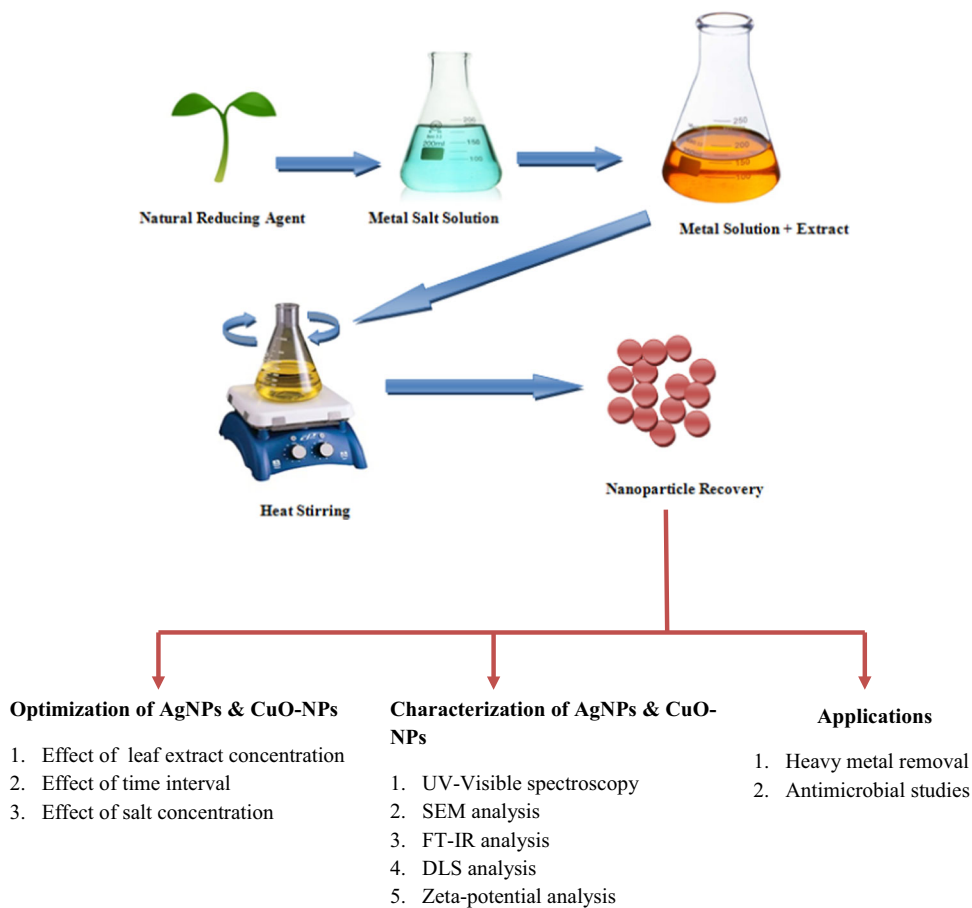


Fig. 2 Schematic representation mechanism of nanoparticle formation

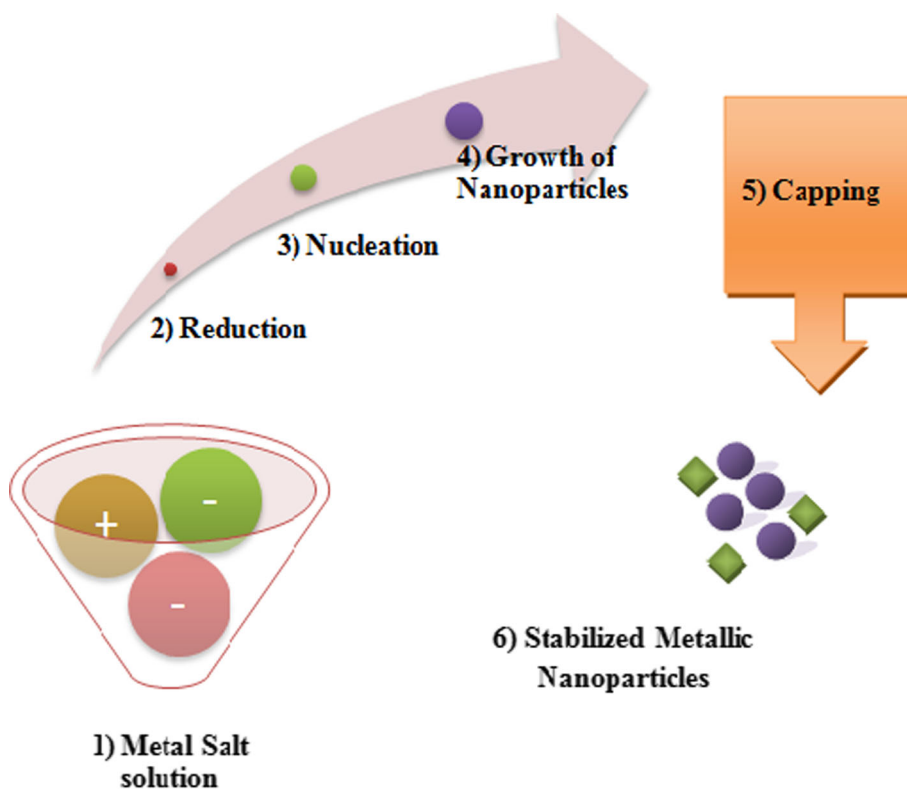


Fig. 3 a) Colour change in 5 mM AgNO_3 after formation of AgNPs b) Colour change in 5 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ after formation of CuO-NPs

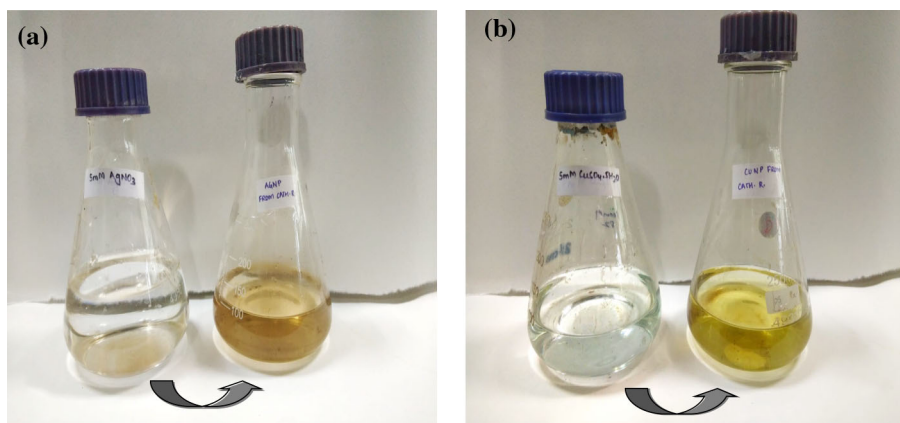
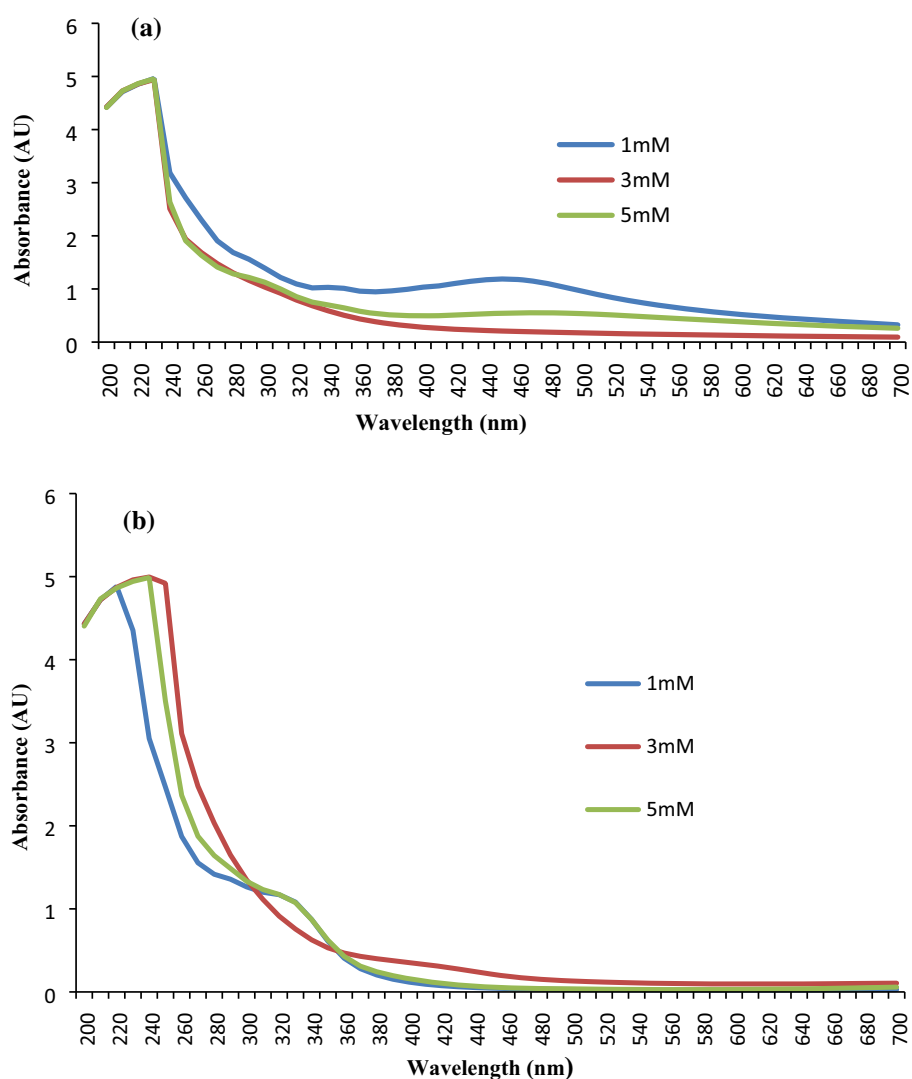


Fig. 4 a) Effect of silver nitrate concentration on AgNP synthesis b) Effect of copper sulfate concentration on CuO-NP synthesis

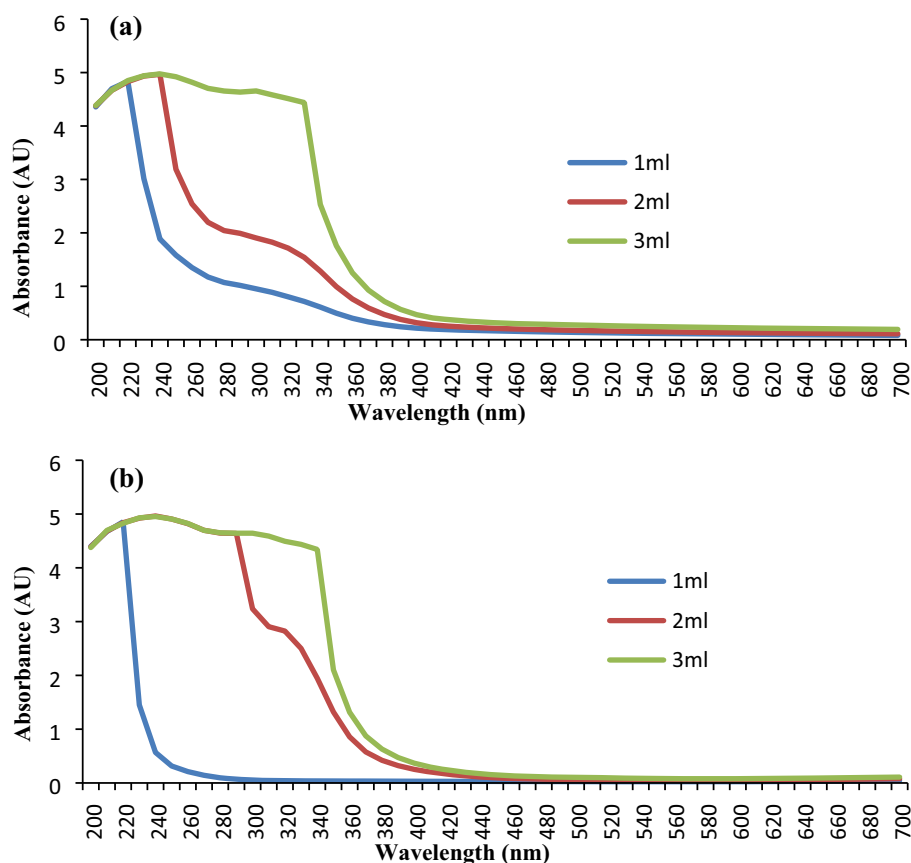


than copper nanoparticle solution. Thus, we conclude that AgNPs have better yield in the process. Similar observations were made by Ruparelia et al. [54]

Scanning Electron Microscope (SEM) Analysis

SEM images were taken at different resolutions i.e.; 200 nm, 300 nm and 1 μm . Silver nanoparticle size ranged

Fig. 5 a) Effect of leaf extract concentration on AgNP synthesis b) Effect of leaf extract concentration on CuO-NP synthesis



from 58.4 nm- 97.4 nm. Spherical shaped particles were obtained which were evenly distributed (Fig. 7a and b). Copper oxide nanoparticles size ranged from 70.1 nm-99.3 nm. They were found to be clustered at some points. They were also uneven and irregular in shape (Fig. 7c and d). The images confirm that copper oxide nanoparticles were comparatively larger than silver nanoparticles. 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. SEM provided details of morphology of synthesized silver and copper oxide nanoparticles which were comparable to findings of Ghiuta et al. [55], Hemmati et al., [56] and Hasheminya and Dehghan-ya.,[57]. The clustering of nanoparticles could be the outcome of bonding between capping organic compounds [58]. A study reported that nanoparticles with size below 5 nm can easily penetrate into tissues and have better renal clearance. Spherical and rod shaped nanoparticles are better taken up by the cell thus making shape and size of nanoparticle an important factor affecting its bioactivity. [59]

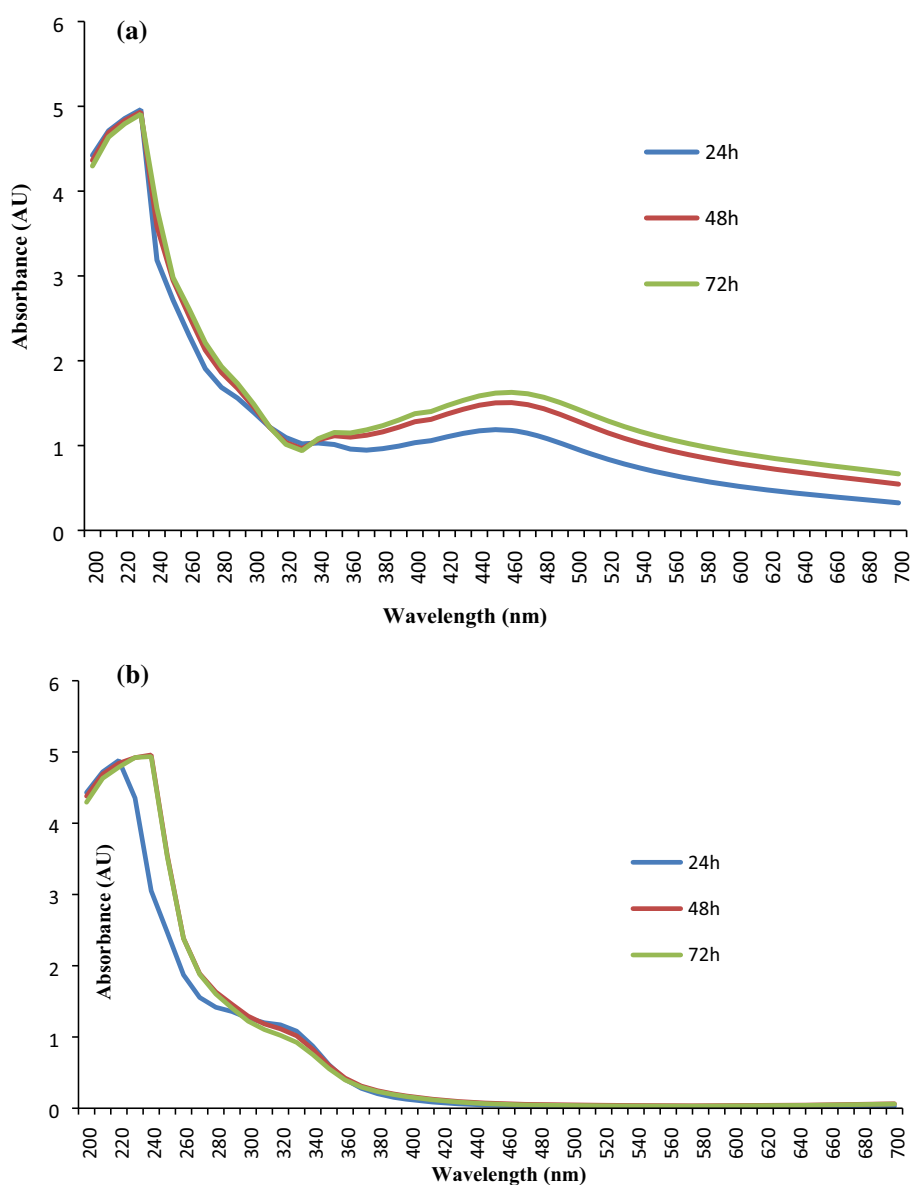
FT-IR, DLS and Zeta-Potential Analysis

Another parameter that can influence that can influence biological activity of plant mediated MNPs is their functionalization [59]. For that purpose FT-IR (Bruker) analysis was done to determine molecular vibrational and organic groups that actively participate in the bio-reduction process of Ag^+ and Cu^{2+} . Identification of capping agents was possible with the help of FT-IR. The analysis was done in different regions of absorbance between 3500 cm^{-1} - 1000 cm^{-1} . IR spectra of silver nanoparticles presented a narrow peak at 3315.91 cm^{-1} that corresponded to $-\text{OH}$ group (alcohol). Other peaks obtained were 2804.66 cm^{-1} , 2155.54 cm^{-1} and 1636.37 cm^{-1} which corresponded to $-\text{CH}$ (alkane), $\text{C}\equiv\text{C}$ (alkyne) and $-\text{NH}_2$ (primary and secondary amines) respectively (Fig. 8a).

IR spectra of copper showed a wide peak at 3303.19 cm^{-1} specifying existence of $-\text{OH}$ bridge (acid). The other two peaks were obtained at 2889.86 cm^{-1} and 1636.26 cm^{-1} correspond to the fact that $\text{C}-\text{H}$ (alkane) and $\text{C}=\text{O}$ (aldehyde, ketone, ester or carboxylic acid) respectively, are present (Fig. 8b).

Nilavukkarasi et al. [60] investigations showed a comparable result. Parallel peak was obtained in case of *R. tuberosa* synthesized CuONPs which represented the OH

Fig. 6 a) Effect of time interval on AgNP synthesis b) Effect of time interval on CuO-NP synthesis



bridge (carboxylic acids) [61]. Peak at 1636.26 resonates with gold nanoparticle peak which represented C = O stretch in a study by [62]. FT-IR analysis for AgNP, CuO-NP and ZnNP were also done by Vijayakumari et al. [63]. Occurrence of aldehydes, alkyne, alkanes carboxylic acids and ketonic groups substantiate the fact that phytochemicals from leaf extract act as capping agents for stabilizing nanoparticles Długosz, et al. [64].

DLS uses variation of time of scattered light from suspended particles which are under Brownian motion. It helps in obtaining hydrodynamic radius (Rh) of particles [26]. Three different peaks were obtained 567 nm, 156.8 nm, 5185 nm and the mean size distribution of silver nanoparticles was 602.9 nm. Polydispersity index (PDI) recorded was 0.835 and intensity of the peak was 62.1% (Fig. 9a). Average size distribution for copper oxide

nanoparticles was 1066 nm with peaks at 901.4 nm, 219.0 nm and 101.8 nm. Polydispersity index (PDI) recorded was 0.648 and intensity of the peak was 74.5% (Fig. 9b).

DLS analysis also indicated that the the average diameter in case of copper was higher than that of silver. Osibe and Aoyagi. [62] also stated that average size determined by DLS is always greater than SEM or TEM because of binding of bio-organic compounds to the core of nanoparticles which does not happen in case of microscopy. Increased size of AgNPs and CuO-NPs could also be due to the layering water molecules in sample [65].

Zeta-potential and zeta-deviation of AgNP was -16.4 mV and 3.94 mV respectively (Fig. 10a). Zeta-potential and zeta-deviation recorded for copper was -6.18 mV and 3.40 mV respectively (Fig. 10b). Both the

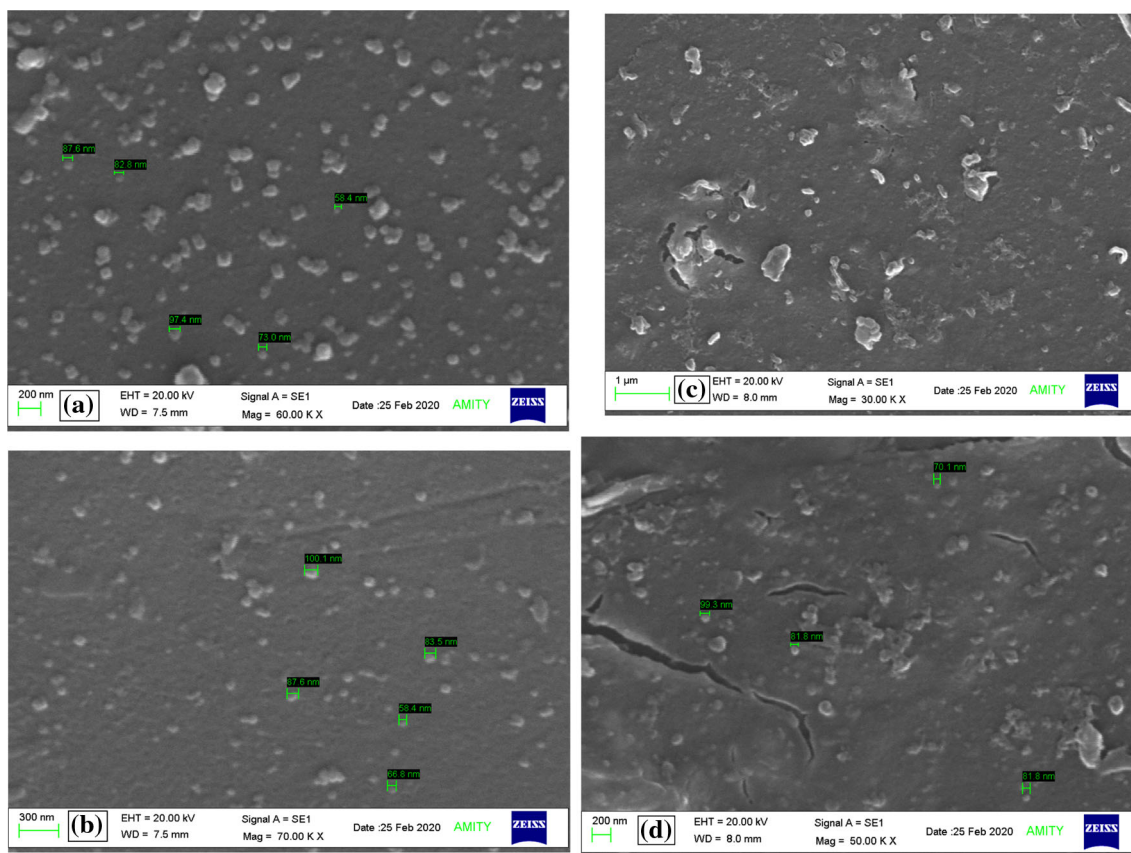


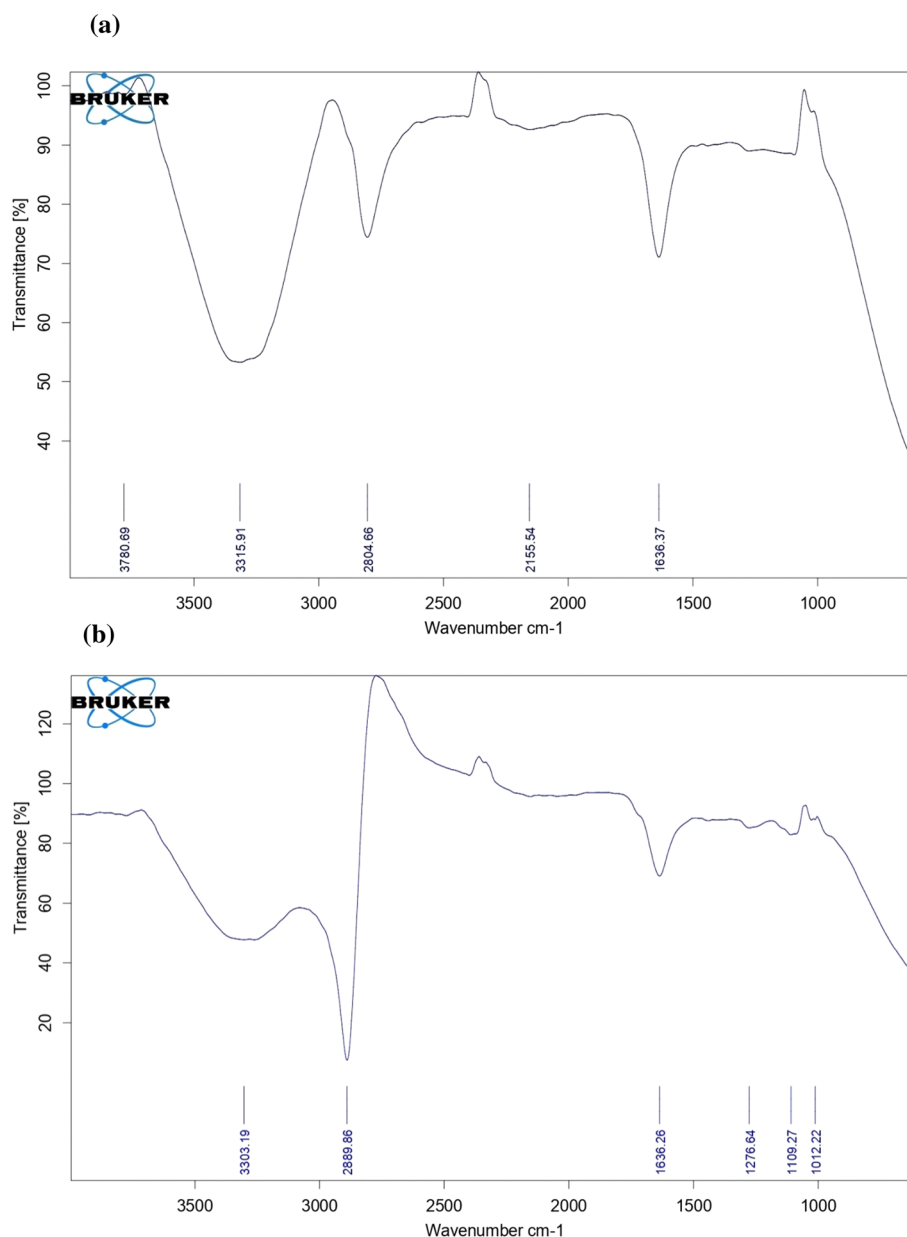
Fig. 7 a) SEM image of silver nanoparticles at 200 nm resolution b) SEM image of silver nanoparticles at 300 nm resolution c) SEM image of copper oxide nanoparticles at 1 μm resolution d) SEM image of copper oxide nanoparticles at 200 nm resolution

particles do not have long term stability and can experience rapid agglomeration unless they are sterically protected. Since the surface charge determination of liquid nanoparticle solutions was unsatisfactory electrical potential of particle was studied. The potential taken at the shear plane is called zeta potential. It helped in determining the surface morphology, stability and surface adsorption studies of suspended particle [66]. The samples were characterized using Malvern Panalytical instrument. The results were analogous to results obtained from *Melia azedarach* [67]. Thirumagal and Jeyakumari [68] stated that negative zeta potential of nanoparticle is a result of adsorption of OH^- ions. A study also suggested that positively charged nanoparticles bind cell membrane effectively and negatively charged nanoparticles avoid NP internalization due to electrostatic repulsion [59]. Jahan et al. [69] in his observations concluded that higher values of zeta potential prevents agglomeration of particles because of repulsion between similarly charged ions.

Heavy Metal Removal Potential

Maximum heavy metal removal efficiency was observed with AgNPs for chromium with a removal efficiency of 47.84%. Cadmium removal efficiency was 5.68%. Removal efficiency for CuO-NP for chromium and cadmium was observed to be 2.11% and 2.91% respectively (Fig. 11). Overall both metallic nanoparticles are effective against cadmium and chromium. But the results also reveal that AgNPs have far superior activity that copper in case of chromium removal. Former had a better performance in case of cadmium as well. Dlamini et al. [70] Samrot et al. [71] support our findings. Investigations have revealed that heavy metal removal by silver and copper oxide nanoparticles is a result of adsorption phenomenon. The large surface area of nanoparticles provide bigger surface area for cadmium and chromium to be adsorbed [44]. Chromium and other heavy metals are adsorbed onto the surface of metallic nanoparticles and are reduced. Mechanism of action these biogenic particles are explained in previous literature [72]. Marimuthu et al., [73] in their literature gave evidences of water treatment by metallic nanoparticles. The removal chromium using heavy metals have been

Fig. 8 a) FT-IR spectrum of silver nanoparticles b) FT-IR spectrum of copper oxide nanoparticles



discussed by Kumari and Tripathi [74]. Heavy metal removal efficiencies using plant mediated MNPs are also dependent upon contact time and pH [75]. These nanoparticles can also be surface coated on membranes for water treatment [76]. Langmuir and Freundlich isotherm model studies for heavy metal remediation have been elucidated by Kumar et al. [77].

Antibacterial Potential

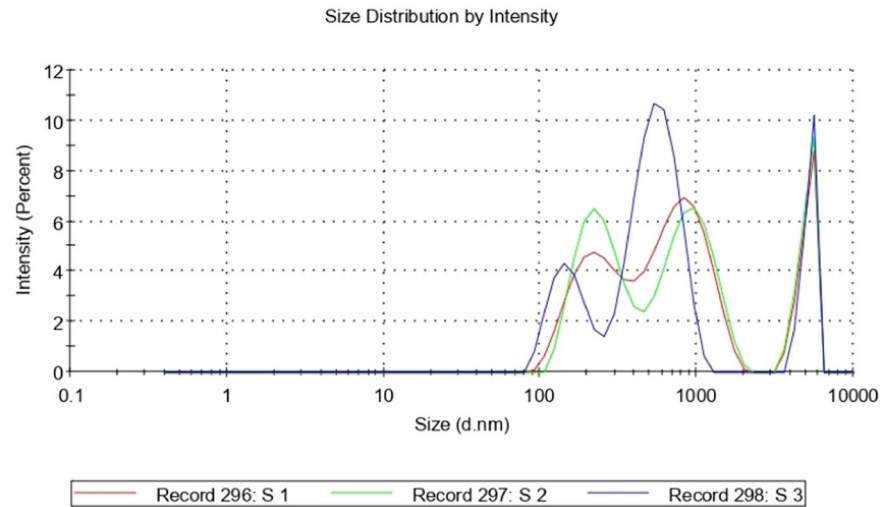
Biosynthesized copper and silver nanoparticles were studied for their bacteriocidal properties using standard microbiology assay of zone of inhibition (ZOI). For the experiment disc diffusion technique was used. Test was

performed against gram positive bacterial *Staphylococcus aureus*. Control drug streptomycin of 10 mg/ml concentration was used. Different concentrations of silver and copper oxide nanoparticles were used. Figure 12a) indicates activity of biogenic AgNPs on *S. aureus*. Inhibitory regions measured for 10 μ l, 30 μ l and 50 μ l concentrations of biogenic AgNP was 7 ± 0.2 , 14 ± 0.2 and 16 ± 0.2 mm respectively. Figure 12b) indicates activity of biogenic CuO-NPs on *S. aureus*. Readings recorded for 10 μ l, 30 μ l and 50 μ l concentrations of biogenic CuO-NP was 5 ± 0.2 , 8 ± 0.2 and 10 ± 0.2 mm respectively. Results clearly show that AgNPs have a tremendous antibacterial potential and perform better than CuO-NPs as well as control drug streptomycin. Antibacterial activity

Fig. 9 a) Size distribution of silver nanoparticles b) Size distribution graph for copper oxide nanoparticles

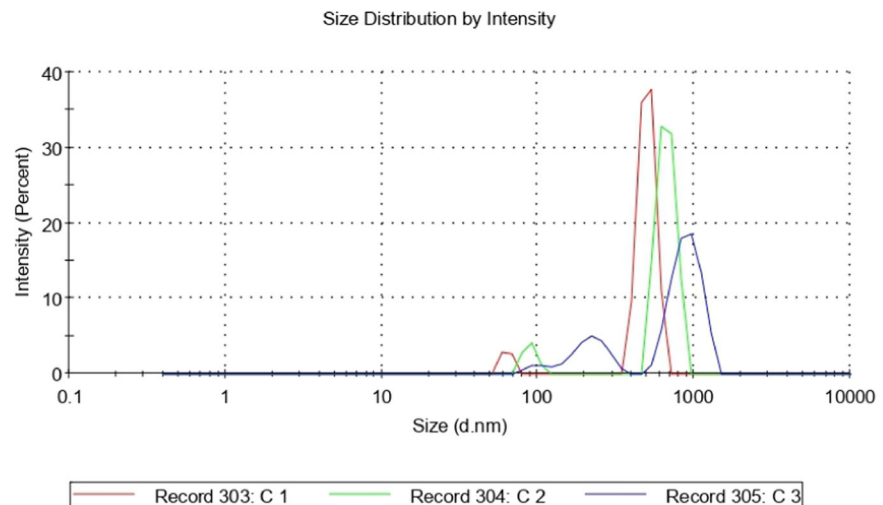
(a)	Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 602.9	Peak 1: 567.0	62.1	179.9
Pdl: 0.835	Peak 2: 156.8	20.8	43.06
Intercept: 0.813	Peak 3: 5185	17.0	483.0

Result quality Refer to quality report



(b)	Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 1066	Peak 1: 901.4	74.5	186.5
Pdl: 0.648	Peak 2: 219.0	21.8	52.74
Intercept: 0.978	Peak 3: 101.8	3.7	14.87

Result quality Refer to quality report

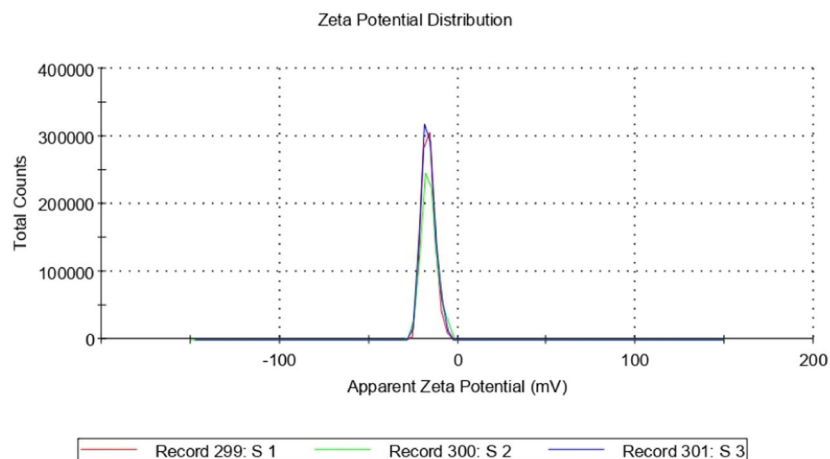


is dependent upon surface to volume ratio of nanoparticles. Biogenic AgNPs have smaller size and spherical shape while CuO-NPs have irregular morphology and comparatively larger size. This makes silver nanoparticles more

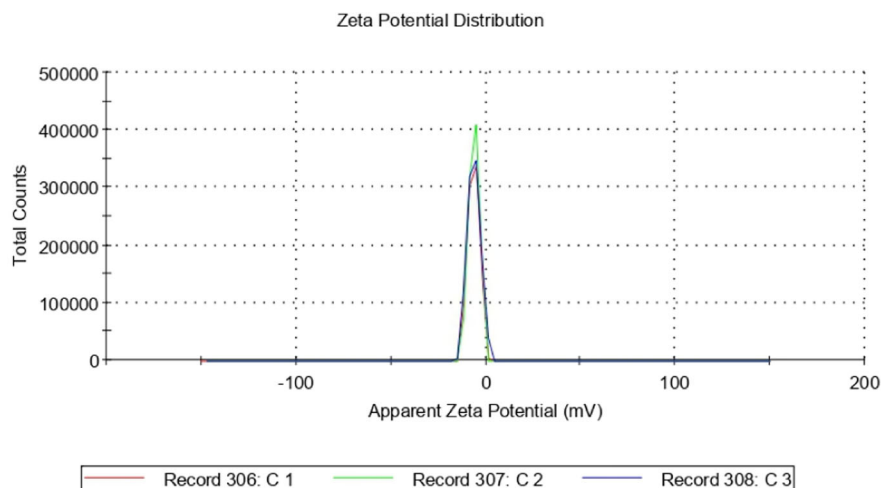
potent than copper [78]. Polyphenols present in leaf extract have bacteria killing properties. Nanoparticles bind to these polyphenolic compounds. Since nanoparticles are extremely small sized particles and have a large surface area,

Fig. 10 a) Zeta-potential of silver nanoparticles b) Zeta-potential of copper oxide nanoparticles

	Mean (mV)	Area (%)	St Dev (mV)
(a) Zeta Potential (mV): -16.4	Peak 1: -16.4	100.0	3.94
Zeta Deviation (mV): 3.94	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.443	Peak 3: 0.00	0.0	0.00
Result quality Good			



	Mean (mV)	Area (%)	St Dev (mV)
(b) Zeta Potential (mV): -6.18	Peak 1: -6.18	100.0	3.40
Zeta Deviation (mV): 3.40	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.429	Peak 3: 0.00	0.0	0.00
Result quality Good			



they act as vehicles for those polyphenols. Reaching the target bacteria using nanoparticles they rupture the protoplasmic membrane and eventually cause cell death [79]. Biogenic silver and copper oxide NPs have been used for their antimicrobial potential in the past. Rajeshkumar et al. [80] synthesized CuO-NPs from *Cissus arnotiana*. *Azadirachta indica* and *Coriandrum sativum* have also been used for the production of AgNPs and CuO-NPs [81]. In a research conducted by Phan et al. [82] comparative

antibacterial testing was done where silver had a better bacteriocidal effect against *E.coli* while copper gave better results against *B. Subtilis*. Silver nanoparticles have previously shown good bacteriocidal effect against *Kleibsellia*, *Shigella*, *P. aeruginosa* and *Bacillus anthracis* [83].

Recent studies on potential of biogenic silver and copper oxide nanoparticles against bacteria have been done by Bindhu et al., Rahmal et al., Maghimaa and Alharbi. [84–86].

Fig. 11 Heavy metal removal efficiency of AgNPs and CuO-NPs

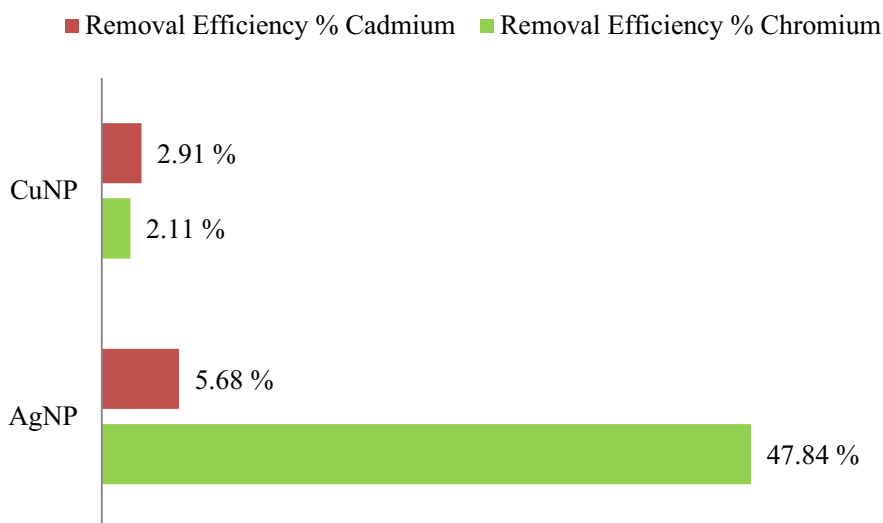
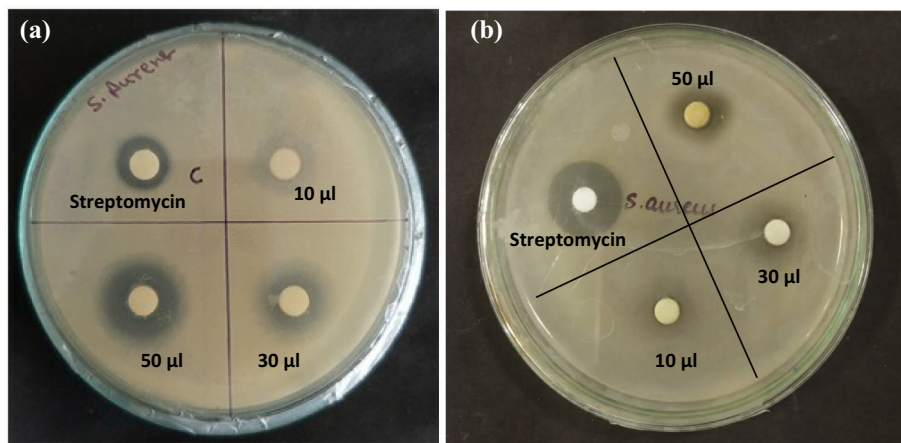


Fig. 12 a) Effect of AgNP on *S. aureus* b) Effect of CuO-NP on *S. aureus*



The antibacterial activity of AgNP and CuO-NP synthesized from *C. roseus* is given below in Table 1.

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. The

morphology and number of nanoparticles synthesized are affected by various conditions. These conditions are time, pH, temperature, metal salt conc., concentration of plant extract. Yield of silver nanoparticle was better than copper oxide 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. Silver and copper oxide nanoparticles both have bacteriocidal effect against *S. aureus*. AgNPs results reveal a larger ZOI than CuO-NPs.

Table 1 Antibacterial testing using Zone of inhibition analysis

Microbe	Nanoparticle	Control (Streptomycin) ZOI (mm)	Zone of Inhibition (mm)		
			10 µl	30 µl	50 µl
<i>Staphylococcus aureus</i>	AgNP	11	7	14	16
	CuO-NP	13	5	8	10

Apparently the synthesis of both nanoparticles cost the same therefore, this establishes silver nanoparticles superior than copper oxide nanoparticles.

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References

- H. Bar, D. K. Bhui, G. P. Sahoo, P. Sarkar, S. P. De, and A. Misra (2009). Green synthesis of silver nanoparticles using latex of *Jatropha curcas*. *Colloids and surfaces A: Physicochemical and engineering aspects* **339** (1–3), 134–139.
- N. N. Nassar, L. A. Arar, N. N. Marei, M. M. A. Ghanim, M. S. Dwekat, and S. H. Sawalha (2014). Treatment of olive mill based wastewater by means of magnetic nanoparticles: Decolourization, dephenolization and COD removal. *Environmental Nanotechnology, Monitoring & Management* **1**, 14–23.
- M. Bundschuh, J. Filser, S. Lüderwald, M. S. McKee, G. Metreveli, G. E. Schaumann, and S. Wagner (2018). Nanoparticles in the environment: where do we come from, where do we go to? *Environmental Sciences Europe* **30** (1), 1–17.
- F. E. Löffler and E. A. Edwards (2006). Harnessing microbial activities for environmental cleanup. *Current Opinion in Biotechnology* **17** (3), 274–284.
- S. Sunkar and C. V. Nachiyar (2012). Microbial synthesis and characterization of silver nanoparticles using the endophytic bacterium *Bacillus cereus*: a novel source in the benign synthesis. *Global J. Med. Res* **12** (2), 43–50.
- P. Khandel and S. K. Shahi (2018). Mycogenic nanoparticles and their bio-prospective applications: current status and future challenges. *Journal of Nanostructure in Chemistry* **8** (4), 369–391.
- A. R. G. Ghomi, M. Mohammadi-Khanaposhti, H. Vahidi, F. Kobarfard, M. A. S. Reza, and H. Barabadi (2019). Fungus-mediated extracellular biosynthesis and characterization of zirconium nanoparticles using standard penicillium species and their preliminary bactericidal potential: a novel biological approach to nanoparticle synthesis. *Iranian journal of pharmaceutical research: IJPR* **18** (4), 2101.
- B. K. Nayak, A. Nanda, and V. Prabhakar (2018). Biogenic synthesis of silver nanoparticle from wasp nest soil fungus, *Penicillium italicum* and its analysis against multi drug resistance pathogens. *Biocatalysis and agricultural biotechnology* **16**, 412–418.
- A. Boroumand Moghaddam, F. Namvar, M. Moniri, S. Azizi, and R. Mohamad (2015). Nanoparticles biosynthesized by fungi and yeast: a review of their preparation, properties, and medical applications. *Molecules* **20** (9), 16540–16565.
- Baker, S., Harini, B. P., Rakshith, D., & Satish, S. (2013). Marine microbes: invisible nanofactories. *journal of pharmacy research*, *6*(3), 383–388.
- R. R. R. Kannan, R. Arumugam, D. Ramya, K. Manivannan, and P. Anantharaman (2013). Green synthesis of silver nanoparticles using marine macroalga *Chaetomorpha linum*. *Applied Nanoscience* **3** (3), 229–233.
- M. I. Din, A. G. Nabi, A. Rani, A. Aihetasham, and M. Mukhtar (2018). Single step green synthesis of stable nickel and nickel oxide nanoparticles from *Calotropis gigantea*: Catalytic and antimicrobial potentials. *Environmental Nanotechnology, Monitoring & Management* **9**, 29–36.
- M. S. Akhtar, J. Panwar, and Y. S. Yun (2013). Biogenic synthesis of metallic nanoparticles by plant extracts. *ACS Sustainable Chemistry & Engineering* **1** (6), 591–602.
- V. Gopinath, D. MubarakAli, S. Priyadarshini, N. M. Priyadarshini, N. Thajuddin, and P. Velusamy (2012). Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: a novel biological approach. *Colloids and Surfaces B: Biointerfaces* **96**, 69–74.
- D. MubarakAli, N. Thajuddin, K. Jeganathan, and M. Gunasekaran (2011). Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. *Colloids and Surfaces B: Biointerfaces* **85** (2), 360–365.
- M. Jeyaraj, M. Rajesh, R. Arun, D. MubarakAli, G. Sathishkumar, G. Sivanandhan, and A. Ganapathi (2013). An investigation on the cytotoxicity and caspase-mediated apoptotic effect of biologically synthesized silver nanoparticles using *Podophyllum hexandrum* on human cervical carcinoma cells. *Colloids and Surfaces B: Biointerfaces* **102**, 708–717.
- S. Priyadarshini, A. Mainal, F. Sonsudin, R. Yahya, A. A. Alyousef, and A. Mohammed (2020). Biosynthesis of TiO₂ nanoparticles and their superior antibacterial effect against human nosocomial bacterial pathogens. *Research on Chemical Intermediates* **46** (2), 1077–1089.
- J. Premkumar, T. Sudhakar, A. Dhakal, J. B. Shrestha, S. Krishnakumar, and P. Balashanmugam (2018). Synthesis of silver nanoparticles (AgNPs) from cinnamon against bacterial pathogens. *Biocatalysis and agricultural biotechnology* **15**, 311–316.
- M. K. Rai, S. D. Deshmukh, A. P. Ingle, and A. K. Gade (2012). Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. *Journal of applied microbiology* **112** (5), 841–852.
- J. R. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri, J. T. Ramírez, and M. J. Yacaman (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology* **16** (10), 2346.
- S. Raina, A. Roy, and N. Bharadvaja (2020). Degradation of dyes using biologically synthesized silver and copper nanoparticles. *Environmental Nanotechnology, Monitoring & Management* **13**, 100278.
- R. Hong, G. Han, J. M. Fernández, B. J. Kim, N. S. Forbes, and V. M. Rotello (2006). Glutathione-mediated delivery and release using monolayer protected nanoparticle carriers. *Journal of the American Chemical Society* **128** (4), 1078–1079.
- R. Pandey and G. K. Khuller (2007). Nanoparticle-based oral drug delivery system for an injectable antibiotic–streptomycin. *Chemotherapy* **53** (6), 437–441.
- J. L. Elechiguerra, J. L. Burt, J. R. Morones, A. Camacho-Bragado, X. Gao, H. H. Lara, and M. J. Yacaman (2005). Interaction of silver nanoparticles with HIV-1. *Journal of nanobiotechnology* **3** (1), 1–10.
- Barabadi, H., Vahidi, H., Mahjoub, M. A., Kosar, Z., Kamali, K. D., Ponmurugan, K., ... & Saravanan, M. (2019). Emerging antineoplastic gold nanomaterials for cervical Cancer therapeutics: a systematic review. *Journal of Cluster Science*, 1–12.
- A. Khatua, A. Prasad, E. Priyadarshini, A. K. Patel, A. Naik, M. Saravanan, and R. Meena (2020). Emerging antineoplastic plant-based gold nanoparticle synthesis: a mechanistic exploration of their anticancer activity toward cervical cancer cells. *Journal of Cluster Science* **31** (6), 1329–1340.
- H. Barabadi, M. Najafi, H. Samadian, A. Azarnezhad, H. Vahidi, M. A. Mahjoub, and A. Ahmadi (2019). A systematic review of the genotoxicity and antigenotoxicity of biologically synthesized metallic nanomaterials: are green nanoparticles safe enough for clinical marketing? *Medicina* **55** (8), 439.

28. Mortezaee, K., Najafi, M., Samadian, H., Barabadi, H., Azarnezhad, A., & Ahmadi, A. (2019). Redox interactions and genotoxicity of metal-based nanoparticles: A comprehensive review. *Chemico-biological interactions*, *312*, 108814.
29. M. C. DeRosa, C. Monreal, M. Schnitzer, R. Walsh, and Y. Sultan (2010). Nanotechnology in fertilizers. *Nature nanotechnology* **5** (2), 91–91.
30. F. Torney, B. G. Trewyn, V. S. Y. Lin, and K. Wang (2007). Mesoporous silica nanoparticles deliver DNA and chemicals into plants. *Nature nanotechnology* **2** (5), 295–300.
31. S. Liu, D. Leech, and H. Ju (2003). Application of colloidal gold in protein immobilization, electron transfer, and biosensing. *Analytical letters* **36** (1), 1–19.
32. L. Gao, D. Zhang, and M. Chen (2008). Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. *Journal of Nanoparticle Research* **10** (5), 845–862.
33. P. R. Aranda, I. Llorens, E. Perino, I. De Vito, and J. Raba (2016). Removal of arsenic (V) ions from aqueous media by adsorption on multiwall carbon nanotubes thin film using XRF technique. *Environmental Nanotechnology, Monitoring & Management* **5**, 21–26.
34. A. Ali, A. Mannan, I. Hussain, I. Hussain, and M. Zia (2018). Effective removal of metal ions from aqueous solution by silver and zinc nanoparticles functionalized cellulose: isotherm, kinetics and statistical supposition of process. *Environmental Nanotechnology, Monitoring & Management* **9**, 1–11.
35. Kumar, L., & Bharadvaja, N. (2019). Enzymatic bioremediation: a smart tool to fight environmental pollutants. In *Smart Bioremediation Technologies* (pp. 99–118). Academic Press
36. S. J. Hawkes (1997). What is a “heavy metal”? *Journal of Chemical Education* **74** (11), 1374.
37. Kumar, L., & Bharadvaja, N. (2020). Microbial Remediation of Heavy Metals. In *Microbial Bioremediation & Biodegradation* (pp. 49–72). Springer, Singapore.
38. M. Ajaib, Z. Khan, N. A. S. R. U. L. L. A. H. Khan, and M. Wahab (2010). Ethnobotanical studies on useful shrubs of district Kotli, Azad Jammu & Kashmir. *Pakistan. Pak. J. Bot* **42** (3), 1407–1415.
39. R. R. Chattopadhyay, S. K. Sarkar, S. Ganguly, R. N. Banerjee, and T. K. Basu (1991). Hypoglycemic and antihyperglycemic effect of leaves of *Vinca rosea* linn. *Indian Journal of Physiology and Pharmacology* **35** (3), 145–151.
40. P. J. Patil and J. S. Ghosh (2010). Antimicrobial activity of *Catharanthus roseus*—a detailed study. *British Journal of Pharmacology and Toxicology* **1** (1), 40–44.
41. Sekar, P. (1996). Vedic clues to memory enhancer. *The Hindu, March*, 21.
42. A. Roy and N. Bharadvaja (2017). Silver nanoparticles synthesis from a pharmaceutically important medicinal plant *Plumbago zeylanica*. *MOJ Bioequiv Availab* **3** (5), 00046.
43. A. Roy (2017). Synthesis of silver nanoparticles from medicinal plants and its biological application: a review. *Res Rev Biosci* **12** (4), 138.
44. Verma, A., Roy, A., & Bharadvaja, N. (2021). Remediation of heavy metals using nanophytoremediation. In *Advanced oxidation processes for effluent treatment plants* (pp. 273–296). Elsevier.
45. H. Barabadi, K. D. Kamali, F. J. Shoushtari, B. Tajani, M. A. Mahjoub, A. Alizadeh, and M. Saravanan (2019). Emerging theranostic silver and gold nanomaterials to combat prostate cancer: a systematic review. *Journal of Cluster Science* **30** (6), 1375–1382.
46. H. Vahidi, H. Barabadi, and M. Saravanan (2020). Emerging selenium nanoparticles to combat cancer: a systematic review. *Journal of Cluster Science* **31** (2), 301–309.
47. H. Barabadi, H. Vahidi, K. D. Kamali, M. Rashedi, O. Hosseini, A. R. G. Ghomi, and M. Saravanan (2020). Emerging theranostic silver nanomaterials to combat colorectal cancer: a systematic review. *Journal of Cluster Science* **31** (2), 311–321.
48. H. Barabadi, H. Vahidi, K. D. Kamali, M. Rashedi, O. Hosseini, and M. Saravanan (2020). Emerging theranostic gold nanomaterials to combat colorectal cancer: a systematic review. *Journal of Cluster Science* **31** (4), 651–658.
49. H. Barabadi, T. J. Webster, H. Vahidi, H. Sabori, K. D. Kamali, F. J. Shoushtari, and M. Saravanan (2020). Green Nanotechnology-based Gold Nanomaterials for Hepatic Cancer Therapeutics: A Systematic Review. *Iranian Journal of Pharmaceutical Research: IJPR* **19** (3), 3.
50. K. Jadhav, S. Deore, D. Dhamecha, R. Hr, S. Jagwani, S. Jalalpure, and R. Bohara (2018). Phyto-synthesis of silver nanoparticles: characterization, biocompatibility studies, and anticancer activity. *ACS Biomaterials Science & Engineering* **4** (3), 892–899.
51. M. S. Hasnain, M. N. Javed, M. S. Alam, P. Rishishwar, S. Rishishwar, S. Ali, and S. Beg (2019). Purple heart plant leaves extract-mediated silver nanoparticle synthesis: Optimization by Box-Behnken design. *Materials Science and Engineering: C* **99**, 1105–1114.
52. M. Souri, V. Hoseinpour, N. Ghaemi, and A. Shakeri (2019). Procedure optimization for green synthesis of manganese dioxide nanoparticles by *Yucca gloriosa* leaf extract. *International Nano Letters* **9** (1), 73–81.
53. S. V. Kumar, A. P. Bafana, P. Pawar, M. Faltane, A. Rahman, S. A. Dahoumane, and C. S. Jeffryes (2019). Optimized production of antibacterial copper nanoparticles in a microwave-assisted synthesis reaction using response surface methodology. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **573**, 170–178.
54. J. P. Ruparelia, A. K. Chatterjee, S. P. Duttagupta, and S. Mukherji (2008). Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta biomaterialia* **4** (3), 707–716.
55. I. Ghiuă, D. Cristea, C. Croitoru, J. Kost, R. Wenkert, I. Vyrides, and D. Munteanu (2018). Characterization and antimicrobial activity of silver nanoparticles, biosynthesized using *Bacillus* species. *Applied Surface Science* **438**, 66–73.
56. S. Hemmati, A. Rashtiani, M. M. Zangeneh, P. Mohammadi, A. Zangeneh, and H. Veisi (2019). Green synthesis and characterization of silver nanoparticles using *Fritillaria* flower extract and their antibacterial activity against some human pathogens. *Polyhedron* **158**, 8–14.
57. Hasheminya, S. M., & Dehghannya, J. (2019). Green synthesis and characterization of copper nanoparticles using *Eryngium caucasicum* Trautv aqueous extracts and its antioxidant and antimicrobial properties. *Particulate Science and Technology*, 1–8.
58. M. Thiruvengadam, I. M. Chung, T. Gomathi, M. A. Ansari, V. G. Khanna, V. Babu, and G. Rajakumar (2019). Synthesis, characterization and pharmacological potential of green synthesized copper nanoparticles. *Bioprocess and biosystems engineering* **42** (11), 1769–1777.
59. Saravanan, M., Barabadi, H., Ramachandran, B., Venkatraman, G., & Ponnuragan, K. (2019). Emerging plant-based anti-cancer green nanomaterials in present scenario. In *Comprehensive Analytical Chemistry* (Vol. 87, pp. 291–318). Elsevier.
60. M. Nilavukkarasi, S. Vijayakumar, and S. P. Kumar (2020). Biological synthesis and characterization of silver nanoparticles with *Capparis zeylanica* L. leaf extract for potent antimicrobial and anti proliferation efficiency. *Materials Science for Energy Technologies* **3**, 371–376.

61. S. Vasantharaj, S. Sathiyavimal, M. Saravanan, P. Senthilkumar, K. Gnanasekaran, M. Shanmugavel, and A. Pugazhendhi (2019). Synthesis of ecofriendly copper nanoparticles for fabrication over textile fabrics: characterization of antibacterial activity and dye degradation potential. *Journal of Photochemistry and Photobiology B: Biology* **191**, 143–149.
62. D. A. Osibe and H. Aoyagi (2019). A novel strategy for the synthesis of gold nanoparticles with *Catharanthus roseus* cell suspension culture. *Materials Letters* **238**, 317–320.
63. J. Vijayakumari, T. L. S. Raj, A. A. Selvi, R. Glenna, and P. Raja (2019). A comparative study of plant mediated synthesis of silver, copper and zinc nanoparticles from *tiliacora acuminata* (lam.) Hook. F. and their antibacterial activity studies. *Synthesis* **18**, 19–34.
64. O. Dlugosz, J. Chwastowski, and M. Banach (2020). Hawthorn berries extract for the green synthesis of copper and silver nanoparticles. *Chemical Papers* **74** (1), 239–252.
65. Mali, S. C., Dhaka, A., Githala, C. K., & Trivedi, R. (2020). Green synthesis of copper nanoparticles using *Celastrus paniculatus* Willd. leaf extract and their photocatalytic and antifungal properties. *Biotechnology Reports*, e00518
66. A. Kalińska, S. Jaworski, M. Wierzbicki, and M. Gołębiewski (2019). Silver and copper nanoparticles —An alternative in future mastitis treatment and prevention? *International journal of molecular sciences* **20** (7), 1672.
67. Jebiril, S., Jenana, R. K. B., & Dridi, C. (2020). Green synthesis of silver nanoparticles using *Melia azedarach* leaf extract and their antifungal activities: In vitro and in vivo. *Materials Chemistry and Physics*, 248, 122898.
68. Thirumagal, N., & Jeyakumari, A. P. (2020). Structural, optical and antibacterial properties of green synthesized silver nanoparticles (AgNPs) using *justicia adhatoda* L. leaf extract. *Journal of Cluster Science*, 31(2), 487–497.
69. Jahan, I., Erci, F., Cakir-Koc, R., & Isildak, I. (2020). Microwave-irradiated green synthesis of metallic silver and copper nanoparticles using fresh ginger (*Zingiber officinale*) rhizome extract and evaluation of their antibacterial potentials and cytotoxicity. *Inorganic and Nano-Metal Chemistry*, 1–11.
70. Dlamini, N. G., Basson, A. K., & Pullabhotla, V. S. R. (2020). Biosynthesis of biofloculant passivated copper nanoparticles, characterization and application. *Physics and Chemistry of the Earth, Parts A/B/C*, 102898.
71. A. V. Samrot, J. L. A. Angalene, S. M. Roshini, P. Raji, S. M. Stefi, R. Preethi, and A. Madankumar (2019). Bioactivity and heavy metal removal using plant gum mediated green synthesized silver nanoparticles. *Journal of Cluster Science* **30** (6), 1599–1610.
72. X. Jin, Y. Liu, J. Tan, G. Owens, and Z. Chen (2018). Removal of Cr (VI) from aqueous solutions via reduction and absorption by green synthesized iron nanoparticles. *Journal of Cleaner Production* **176**, 929–936.
73. Marimuthu, S., Antonisamy, A. J., Malayandi, S., Rajendran, K., Tsai, P. C., Pugazhendhi, A., & Ponnusamy, V. K. (2020). Silver nanoparticles in dye effluent treatment: A review on synthesis, treatment methods, mechanisms, photocatalytic degradation, toxic effects and mitigation of toxicity. *Journal of Photochemistry and Photobiology B: Biology*, 205, 111823.
74. Kumari, V., & Tripathi, A. K. (2020). Remediation of heavy metals in pharmaceutical effluent with the help of *Bacillus cereus*-based green-synthesized silver nanoparticles supported on alumina. *Appl. Nanosci.*
75. M. A. Ahmed, S. T. Bishay, F. M. Ahmed, and S. I. El-Dek (2017). Effective Pb 2+ removal from water using nanozerovalent iron stored 10 months. *Applied Nanoscience* **7** (7), 407–416.
76. P. Choudhury, P. Mondal, S. Majumdar, S. Saha, and G. C. Sahoo (2018). Preparation of ceramic ultrafiltration membrane using green synthesized CuO nanoparticles for chromium (VI) removal and optimization by response surface methodology. *Journal of Cleaner Production* **203**, 511–520.
77. K. Y. Kumar, H. B. Muralidhara, Y. A. Nayaka, J. Balasubramanyam, and H. Hanumanthappa (2013). Low-cost synthesis of metal oxide nanoparticles and their application in adsorption of commercial dye and heavy metal ion in aqueous solution. *Powder technology* **246**, 125–136.
78. Zia, R., Riaz, M., Farooq, N., Qamar, A., & Anjum, S. (2018). Antibacterial activity of Ag and Cu nanoparticles synthesized by chemical reduction method: a comparative analysis. *Materials Research Express*, 5(7), 075012
79. C. P. Devatha, K. Jagadeesh, and M. Patil (2018). Effect of green synthesized iron nanoparticles by *Azadirachta Indica* in different proportions on antibacterial activity. *Environmental Nanotechnology, Monitoring & Management* **9**, 85–94.
80. Rajeshkumar, S., Menon, S., Kumar, S. V., Tambuwala, M. M., Bakshi, H. A., Mehta, M., ... & Dua, K. (2019). Antibacterial and antioxidant potential of biosynthesized copper nanoparticles mediated through *Cissus arnotiana* plant extract. *Journal of Photochemistry and Photobiology B: Biology*, 197, 111531.
81. Abbas, S., Nasreen, S., Haroon, A., & Ashraf, M. A. (2020). Synthesis of Silver and copper nanoparticles from plants and Application as Adsorbents for Naphthalene Decontamination. *Saudi Journal of Biological Sciences*.
82. D. N. Phan, N. Dorjjugder, M. Q. Khan, Y. Saito, G. Taguchi, H. Lee, and I. S. Kim (2019). Synthesis and attachment of silver and copper nanoparticles on cellulose nanofibers and comparative antibacterial study. *Cellulose* **26** (11), 6629–6640.
83. Ahmad, S., Tauseef, I., Haleem, K. S., Khan, K., Shahzad, M., Ali, M., & Sultan, F. (2019). Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* and their antimicrobial activity. *Applied Nanoscience*, 1–6.
84. Bindhu, M. R., Umadevi, M., Esmail, G. A., Al-Dhabi, N. A., & Arasu, M. V. (2020). Green synthesis and characterization of silver nanoparticles from *Moringa oleifera* flower and assessment of antimicrobial and sensing properties. *Journal of Photochemistry and Photobiology B: Biology*, 205, 111836.
85. A. U. Rahman, A. U. Khan, Q. Yuan, Y. Wei, A. Ahmad, S. Ullah, and W. Ahmad (2019). Tuber extract of *Arisaema flavum* eco-benignly and effectively synthesize silver nanoparticles: Photocatalytic and antibacterial response against multidrug resistant engineered *E. coli* QH4. *Journal of Photochemistry and Photobiology B: Biology* **193**, 31–38.
86. Maghimaa, M., & Alharbi, S. A. (2020). Green synthesis of silver nanoparticles from *Curcuma longa* L. and coating on the cotton fabrics for antimicrobial applications and wound healing activity. *Journal of Photochemistry and Photobiology B: Biology*, 204, 111806.

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