e-planet **17** (1): 76-82 (June 2019)

A comparative study of two different methods for synthesis of copper nanoparticles

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Date of receipt: 25.04.2019 Date of acceptance: 27.06.2019

ABSTRACT

Synthesis of copper nanoparticles was done using green chemistry and green synthesis, using plant leaf extract *Murraya koenigii*. The efficacy of two methods was compared by focussing on some of the parameters like particle size, stability and antibacterial activity. On treatment of aqueous solution of $CuSO_4 \cdot 5H_2O$ with *Murraya koenigii* leaf extract, stable copper nanoparticles were formed. UV-vis spectroscopy was used to monitor the quantitative formation of copper nanoparticles. To 1.2% of aqueous starch solution $CuSO_4 \cdot 5H_2O$ was added in the presence of ascorbic acid as the capping agent. The synthesized nanoparticles were characterized with UV-Vis Spectroscopy and DLS. By DLS analysis of copper nanoparticles particle size was of 133.4 nm for curry leaf extract and 43.42 nm for green chemistry was observed. Stability of green chemistry synthesized copper nanoparticle was more as compared to biosynthesized nanoparticle. Antibacterial tests showed that biosynthesized copper nanoparticles were more effective as compared to green chemistry synthesized nanoparticle. As a possible green alternative to physical and chemical processes, biogenesis of copper nanoparticle using plant extract may have application in various water purification systems.

Key words: antibacterial activity, biogenesis, copper nanoparticles, green chemistry, plant extract (*Murraya koenigii*),

INTRODUCTION

In the last decade nanotechnology is widely used in diverse fields like medicine, electronics, biomaterials, food, agriculture and energy production. An imperative area of research in this field is to explore the synthesis of nanoparticles with different chemical compositions, as well as biosynthesis of metal nanoparticles using plant extracts and microbes. Copper nanoparticles have been synthesized by either physical or chemical methods like mechanochemical process (Subhankari and Nayak, 2013), vacuum vapor deposition, (Liu and Bando, 2003) sonochemical reduction (Kumar et al., 2001), reduction processes like thermal, chemical and direct electrochemical (Dang et al., 2011). Nanoparticles produced by conventional physical or chemical methods, uses many toxic chemicals as capping agent and stabilizing agent, thus restricting its use in clinical and biomedical field. The greener approach of fabrication of nanoparticles using noble and other metals is the more favored one as it is a cleaner, economical and environmental friendly process.

Biological methods for synthesis of copper nanoparticles using plant extracts are simple, inexpensive and eco-friendly. Phytochemicals present in plants like terpenoids, flavonoids, carboxylic acids, quinones, aldehydes, ketones, amides spontaneously reduce metal ions and also control the size and shape of the metal nanoparticle (Prabhu and Poulose, 2012). Two greener approaches are used for synthesis of nanoparticles either green chemistry or green synthesis. Green chemistry focuses on the production of nanoparticles by using certain chemicals that are environmentally friendly and can serve as both capping and reducing agent (Xiong et al., 2011). While green synthesis utilizes plant parts such as roots stems and leaves and its aqueous extracts for synthesis of nanoparticles.

Copper nanoparticles due to their unique physical and chemical properties, low cost of preparation compared to noble metals have been of great interest in nanoscience. Copper nanoparticles (Cu-NPs) have special properties, which have made them important for various applications; for example, super strong materials, antibacterial, sensors and catalysts (Javier et al., 2017). Furthermore, they can also interact and react with other nanoparticles due to the high surface area-volume ratio. Synthesis of CuO nanoparticles has been done using various plant extracts of Tea leaf (Vaseeharan et al., 2010) *Magnolia kobus* (Lee et al., 2013), *Piper longum* (Jacob et al., 2012), *Ocimum sanctum* (Kulkarni and Kulkarni 2013), *Syzygium aromaticum* (Subhankari and Nayak, 2013), *Nerium oleander* (Gopinath et al., 2014), *Carica papaya* (Sankar et al., 2014), *Psidium guajava* (Caroling et al., 2015), *Butea monosperma* (Chaturvedi and Verma, 2015), *Calotropis gigantean* (Behera and Giri, 2016), *Murraya koenigii* (Ashtaputrey et al., 2017). Curry leaves has recently been found to be a potent antioxidant due to high concentrations of carbazoles, a water soluble heterocyclic compound (Rai et al., 2008) which is responsible for the reduction and stabilization of metal ions. Green Synthesis of copper nanoparticle was done using curry leaf extracts.

In contrast to plant extracts copper nanoparticles synthesized by green chemistry uses many surfactants as dispersants, capping and reducing agent like ascorbic acid that prevents oxidation and precipitation of copper nanoparticles in colloidal solution. The current study was undertaken to analyze and compare some physical properties like size, stability and antibacterial activity of Copper Nanoparticles synthesized through green chemistry and green synthesis.

MATERIAL AND METHODS

Synthesis of copper nanoparticles using plant extract

For green synthesis of Copper nanopartcles *Murraya koenigii* (curry leaves) were taken. The leaves were collected and shade dried for 2 days. Then 50 g of leaves were crushed in Mixer and boiled in 500 ml of sterile distill water for 15 minutes. A pale brown colour extract was obtained which was allowed to cool at room temperature and then filtered through Whatman filter paper. To 1mM aqueous $CuSO_4{\bullet}5H_2O$ solution of 10 ml, 5 ml of aqueous leaf extract was added at room temperature. A gradual change in colour from pale yellow to amber colour was seen after 20 hrs (Fig.1a and b).

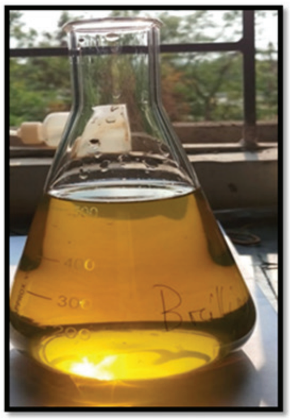

Fig. 1(a)

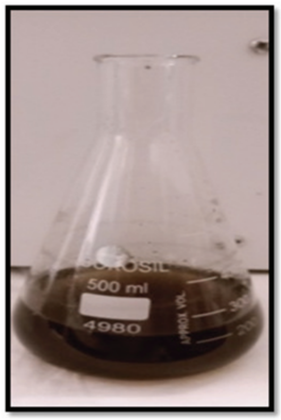

Fig. 1(b)

Fig. 1(a). *Murraya koenigii* (curry leaves) plant extract

Fig. 1(b). Change in colour from yellow to amber colour after addition of plant extract to copper sulphate solution indicating the synthesis of copper nanoparticles

Synthesis of copper nanoparticles by Green chemistry

Copper nanoparticles were synthesized by dissolving 0.1 M copper (II) sulfate pentahydrate solution into 120 mL of starch (1.2 %) solution with vigorous stirring for 30 min. It was followed by addition of 50 mL of 0.1 M ascorbic acid solution to synthesis solution under continuous rapid stirring. The pH of the experiment was maintained at 6.5 by adding aqueous NaOH. The mixture was mixed in a small magnetic stirrer at a constant temperature of 85°C. A gradual change in colour was obtained from white to blue then dark green, orange and dark brown (Fig. 2a,b,c,d and e).

Fig. 2(a). Starch solution (1.2%)

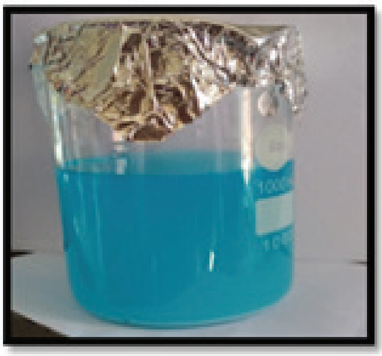

Fig. 2(b). Addition of copper sulphate pentahydrate to Starch solution

Fig. 2(c). Starch copper sulphate and (0.1M) ascorbic acid

Fig. 2(d). Addition of sodium hydroxide and continuous stirring at 80°C

Fig. 2(e). Colour changes from yellow to ocher indicating the synthesis of copper nanoparticles after 24 hrs

Characterization techniques

The synthesized copper nanoparticles were characterized by UV-Vis Spectroscopy and DLS. By using phase analysis light scattering technique zeta potential was measured using a Malvern Nano ZS instrument. Copper nanoparticles synthesized, were measured in aqueous state.

Stability of nanoparticles

Stability of the colloidal solution of copper nanoparticles were checked after 24 hrs of incubation, and then regularly for 15 days.

Test microorganisms

To 1 ml of local municipality supply water, 9 ml of sterile distilled water was added. Then serial dilution was done and 100 µl from each dilution was plated on Nutrient Agar to get the microbial count. Different colored, shaped colonies, large mucilaginous colonies were picked from Nutrient Agar (NA) plates and Gram Staining was done for initial screening. Black centered colonies with greenish metallic sheen were seen on Eosin Methylene Blue (EMB) plates.

Antibacterial Assay

Nutrient Agar and Eosin Methylene Blue petriplates were prepared as per the manufacturer instructions. Using cork-borer 6mm diameter wells were made on NA and EMB plates. On each NA plate, 6.8 x 10^3 colonies were inoculated and in each well 50 µl of copper nanoparticle using green chemistry and green synthesis were added. The plates were incubated at 37°C for 18hrs. After incubation distinct lawn of microorganisms were seen on NA plates and a clear zone was seen around the wells. On EMB plates 50 µl municipality water was spread and in wells 50 µl of copper nanoparticle was added. After overnight incubation the EMB plates were filled with *E. coli* colonies and distinct zones were seen around the wells. The diameter of the zones was measured to study the inhibitory effect of copper nanoparticles on microorganisms.

RESULTS AND DISCUSSION

Recently developed methods for nanoparticles include laser ablation, chemical reduction, polyol synthesis, thermal decomposition and biosynthesis of copper nanoparticles. Among these processes, green chemistry and green synthesis of copper nanoparticles are preferred for their low toxicity and ecofriendliness. In the current study copper nanoparticles particle was synthesized in two different methods; and the effectiveness of CuNPs was compared by investigating various parameters like size and stability. Secondly the antibacterial effect of copper nanoparticles against microbes and Gram negative bacteria was also studied.

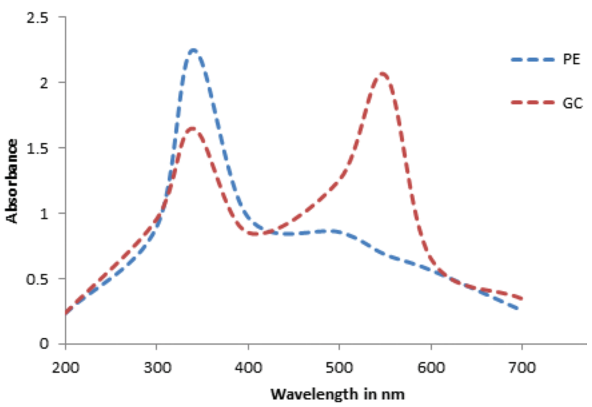

Fig. 3. The UV Visible absorption spectra of copper nanoparticles in plant extract (PE) and UV Visible absorption spectra of copper nanoparticles stabilized in 0.1M ascorbic acid (GC)

Characterization of CuNPs

UV-Vis Spectra

UV- Visible absorbance spectroscopy is the most simple and effective method for studying metal nanoparticles as the peak position and shape depends upon particle size. UV-Visible spectroscopy from a double beam spectrophotometer (U.V. 3000+ LABINDIA, path length 1.0 cm spectral range from 200 nm to 800 nm) was used to study the synthesis of copper nanoparticles.

Curry leaf extract of 5 ml was added to 1mM $CuSO_4$ and a gradual change in colour was seen from yellow to amber colour after 48 hrs. Similar results were obtained by Sankar (2014) working on *C. papaya* leaf extract containing 5mM $CuSO_4$, were colour change begins after 24 hrs of incubation and becomes dark brown after 48hrs of incubation. Copper nanoparticles synthesized by green chemistry initially were orange in colour and gradually the colour changed to dark brown after 24 hr of incubation.

The absorption spectrum pattern of CuNP synthesized using green chemistry was different from the CuNP synthesized using curry leaves. While a single, sharp and prominent peak at 340 nm was observed for CuNP synthesized using curry leaves extract, two prominent peaks were seen for CuNP synthesized using green chemistry. Fig. 3 shows the UV-Vis spectrum of CuNP in aqueous medium of curry leaf extract. The absorption peak corresponds to copper nanoparticle synthesis, and its stability (Gopinath, 2014), which has evolved during the reaction time and the change in colour validates it. Two absorption peaks one at 335 nm corresponding to oxidation product of L-ascorbic acid (Xiong et al., 2011) and a broader peak at 560 nm which confirms the formation of CuNPs (Fig. 3). High absorbance is an indication of faster conversion of copper to copper nanoparticles thus leading to higher concentration of copper nanoparticle in the reaction mixture.

DLS

Dynamic light scattering (DLS) analysis was used to find out the size and surface charge of nanoparticles. CuNP synthesized using plant extract (curry leaves) had particle size of 133.4nm (Fig. 4) while CuNP synthesized using green chemistry 43.42 nm (Fig. 5). Dang (2011) used ethylene glycol to synthesize colloidal copper nanoparticles by a chemical reduction method in water. In both media, the particles were rather spherical, and it has been found that the average diameters of the copper nanoparticles were 22 nm and 10 nm in water and EG, respectively, for the most stable solutions. Hoda (2012) reported synthesis of copper nanoparticles (Cu-NPs) in chitosan (Cts) media via a chemical reaction method using ascorbic acid as an antioxidant with particle sizes in the range of 35–75 nm. Behera and Giri (2015) synthesized cuprous oxide (Cu_2O) nanoparticles (NPs) with an average crystallite size of 8.8 nm in presence of Arka (*Calotropis gigantea*) leaves extract. *Syzygium aromaticum* (Clove) extracts produced Cu nanoparticles with a average particle size of 40 nm and a spherical to granular morphology (Subhankari and Nayak, 2013). Padil (2013) using *Sterculia urens* (Karaya gum) extract was able to synthesize highly stable spherical Copper Oxide nanoparticles with a mean particle size of 4.8 nm.

Stability of copper nanoparticles

Small metal nanoparticles absorb visible electromagnetic waves by oscillation of conduction electrons at the surface (Dang et al., 2011). This is known as Surface Plasmon effect. Stability of the CuNP was measured by observing the absorption peak at the time of complete colour change and

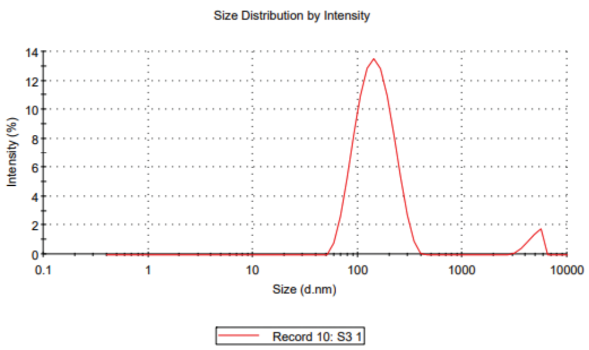

Fig. 4. Average diameter of CuNPs using (PE) is found to be 133.4 nm

then at regular intervals for fifteen days. There was gradual decrease in peak intensity after ten days for copper nanoparticles synthesized using curry leaf extract (Fig. 6). A shift in peak position was also seen for colloidal copper nanoparticles in aqueous medium, with ascorbic acid as capping agent (Fig. 7). The oxidation and precipitation was seen after ten days in case of CuNP synthesized using curry leaf extract while colloidal CuNP synthesized using green chemistry were stable for fifteen days.

Copper nanoparticles synthesized in ambient atmosphere without inert gas intervention have tendency to oxidize to copper oxides as the later are thermodynamically more stable. In the absence of protection, copper nanoparticles tend to aggregate very fast. So in green chemistry method for synthesis of copper nanoparticles, ascorbic acid

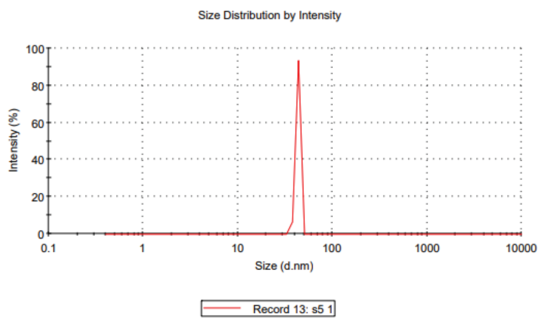

Fig. 5. Average diameter of CuNPs (GC) is found to be 43.42 nm

is used as a reducing and capping agent to avoid contamination by other organic compounds. It has been reported earlier that ascorbic acid reduces aggregation of nanoparticles to great extent (Khan et al., 2016). The bioactivity of curry leaves is attributed to the presence of phytochemicals like phenolic acids, essential oils terpenoids, alkaloids, tocopherol, β-carotene, lutein (Patterson and Verghese, 2015). Though it has been reported that high concentration of carbazole in curry leaves is responsible for reduction and stabilization of metal ions (Sajeshkumar et al., 2015) however the exact mechanism for reduction of copper ions in aqueous solutions is yet to be identified.

Antibacterial activity

Antibacterial activity was tested against *E. coli* and Gram positive bacteria. After overnight

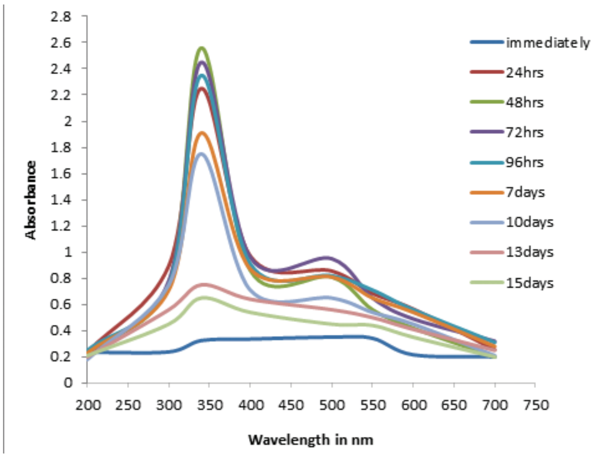

Fig. 6. UV visible absorption spectra of copper nanoparticle synthesized using plant extract for a period of 15 days.

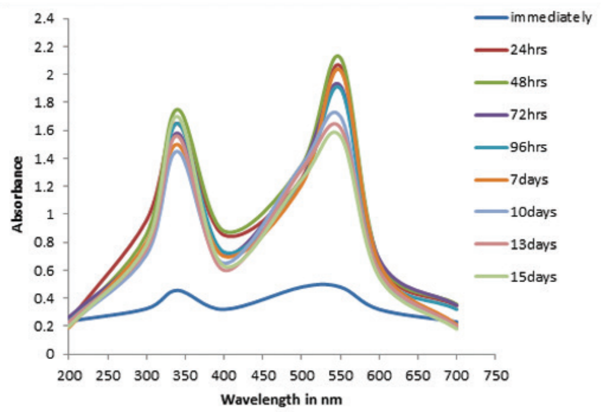

Fig. 7. UV visible absorption spectra of copper nanoparticle synthesized using green chemistry for a period of 15 days.

incubation distinct zones were seen around the wells containing 50 µl of copper nanoparticle. The NA plate containing 6 x 10^3 colonies of bacteria showed a clear zone of 5 mm for CuNP synthesized using Green Chemistry and a bigger zone of 9 mm for CuNP synthesized using plant extract (Fig. 5). In the EMB *E. coli* plate the clear zone size for CuNP synthesized using Green Chemistry was 5.4 mm and 11.2 mm for CuNP synthesized using plant extract.

The biologically synthesized CuNP using plant extract showed higher antibacterial activity compared to CuNP synthesized using ascorbic acid and starch. Similar results were also found by (Lee et al., 2016) working on synthesis of $CuSO_4$ using Magnolia leaf extract and chemically synthesized copper nanoparticles using sodium borohydride and Tween 20. The bactericidal effect of metal nanoparticles is due to their small size and high surface to volume ratio that helps them to interact with the microbial membranes and not due to the release of metal ions into the solution. They have found CuNPs to be effective in controlling growth of *Escherichia coli* cells, a common pathogen. CuNPs synthesized using karaya gum extract showed antimicrobial activity against universal pathogens such as *Escherichia coli* and *Staphylococcus aureus* (Padil et al., 2013)

CONCLUSION

In this study, CuNPs were successfully synthesized by green chemistry and green synthesis methods. Curry leaves extract was utilized as a natural reducing and capping agent in synthesizing CuNPs. Ascorbic acid was the stabilizing agent for green chemistry. Though the stability of CuNPs synthesized using curry leaf extract was less compared to green synthesized CuNP but antibacterial activity was more pronounced for biosynthesized CuNPs. The identification of CuNPs was done by characterization techniques such as DLS and UV-VIS spectroscopy. CuNP synthesized using green chemistry had particle size of 43.42 nm while CuNP synthesized using plant extract (curry leaves) 133.4 nm. The particle size was larger for biosynthesized CuNP which was could be reduced to smaller size by adjusting the concentration of plant extract. Thus in the current study an economical and faster method of biogenesis of copper nanoparticles has been proposed and its effectiveness in controlling growth of bacteria and *E. coli* in water purification systems.

ACKNOWLEDGEMENT

The authors are grateful to Dept. Of Biotechnology of Rama Devi Women's University.

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