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Effect of Diverse Factors on Green Synthesis of Copper Nanoparticles

P. Naga Padma*, **S. Chaitanya Kumari and Syed Thanveer Banu**

Department of Microbiology, Bhavan's Vivekananda College of Science, Humanities and Commerce, Sainikpuri, Secunderabad-94, Telangana State, India.
*Corresponding Author: naga_padmathota@yahoo.com

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ABSTRACT

Metallic nanoparticles like those of copper, silver and gold are of great interest both due to their excellent physical, chemical properties and antibacterial property. The conventional non biological methods for their synthesis are not eco-friendly as they involve use of toxic reagents and also produce harmful by-products. To overcome these limitations, green synthesis of metal nanoparticles was developed and gained research focus as these have significant biomedical applications. Copper nanoparticles due to their interesting properties, low cost preparation and many potential applications in electronics, medical and pharma fields have attracted a lot of interest in recent years. The present study focused on various factors that influenced green synthesis of preferably copper nanoparticles using copper sulphate solution and different plant extracts of *Punica granatum*. The influencing factors studied were over a range like molarity of copper sulphate solution from 1 mM-20mM, incubation time 0-2 hrs, temperature 25^{0}C-55^{0}C, pH 5-11 respectively. The study is significant as standardization of factors for green synthesis of copper nanoparticles can facilitate their bulk synthesis commercially. These copper nanoparticles can be used as antimicrobial agents and help to combat the fast growing drug resistance.

Keywords: Green synthesis, Copper nanoparticles, pH, Temperature, Plant extracts.

INTRODUCTION

Nanotechnology is considered to be the most widely studied field in science that involves the study of manipulation of materials at the atomic level by a combination of engineering, chemical, and biological approaches (Cauerhff *et al*, 2013). Numerous efforts are being made throughout the world to develop ecofriendly technologies to produce environmentally benign, non toxic products using green nanotechnology and biotechnological tools (Joerger *et al*, 2000, Chauhan *et al,* 2012, Syed Thanveer *et al,* 2018).

The green synthesis of copper nanoparticles using modern techniques has gained research prominence due to their application in the biomedical and human health care field (Parashar *et al*, 2009). Copper nanoparticles are preferred as they have greater stability, appropriate dimensions and antibacterial property and can be synthesized using a one-step procedure. Various undesirable processing conditions are thus eliminated by allowing the synthesis to proceed at physiological temperatures, pH at a negligible cost (Kundu *et al* 2008).

Copper is one of the most widely used metal with multiple uses and its nanoparticles also have various applications but their synthesis is challenging due to copper's reactive nature and high tendency for oxidation (Carl *et al* 1967). Unlike gold and silver, copper is extremely sensitive to air, and the oxide phases are thermodynamically more stable (Jeong *et al*, 2008) but the presence of copper oxides on the surface of nanoparticles hinders their application in electronics and biological fields. Taking this aspect into consideration the present study focused on the factors that affect green synthesis of copper nanoparticles.

The quality, type and characteristics of nanoparticles synthesized by green technology are greatly influenced by the incubation time (Rai *et al,* 2006), storage time and conditions (Kuchibhatla *et al,* 2012, Mudunkotuwa *et al*, 2012) as they causes variations in size and number. The variations on storage may occur in many ways such as aggregation, shrinkage which in turn influences their shelf life (Baer, 2011). Temperature is another important parameter that affects the synthesis of nanoparticles and the temperature of the reaction medium determines the nature of the nanoparticle formed. In most cases, the synthesis of nanoparticles using green technology requires mesophilic temperatures. pH of the reaction mixture also influences the shape, size and texture of the synthesized nanoparticles (Gardea-Torresdey *et al*, 1999, Armendariz *et al*, 2004).

MATERIALS AND METHODS

Green synthesis of Copper Nanoparticles

Different aqueous extracts of *Punica granatum* like those of leaf, flower, fruit rind and seed were added to 1mM of aqueous copper sulphate solution in 1:10 ratio. Change in the color of the solution from blue to pale yellow was observed as it indicated copper nanoparticle synthesis. The flasks were incubated overnight at room temperature. Post incubation the copper nanoparticles formed separated out and settled at the bottom of the flask.

Effect of different process parameters on green synthesis of Copper Nanoparticles

Copper sulphate solutions of different molarities like 1mM, 5mM, 10mM and 20mM were mixed with the leaf, fruit rind, seeds and flower extracts of *Punica granatum* (50% concentrated). Post incubation the copper sulphate solution with plant extracts showed effective dark yellow color formation indicating the synthesis of copper nanoparticles (Syed Thanveer Banu *et al,* 2018).

The biosynthesis of copper nanoparticles was carried out at different temperatures namely; 25^0c, 35^0c, 45^0c, 55^0c and pH 5, 7, 9 and 11 with 50% concentrated sample of leaf and fruit rind extracts, and the time taken for the formation was noted for every 30 minutes and completion of the reaction was monitored by the color change as well as the UV- Visible spectrum.

Detection of copper nanoparticles

The green synthesized copper nanoparticles were detected qualitatively by UV-Vis spectroscopy and Scanning electron microscopy (SEM).

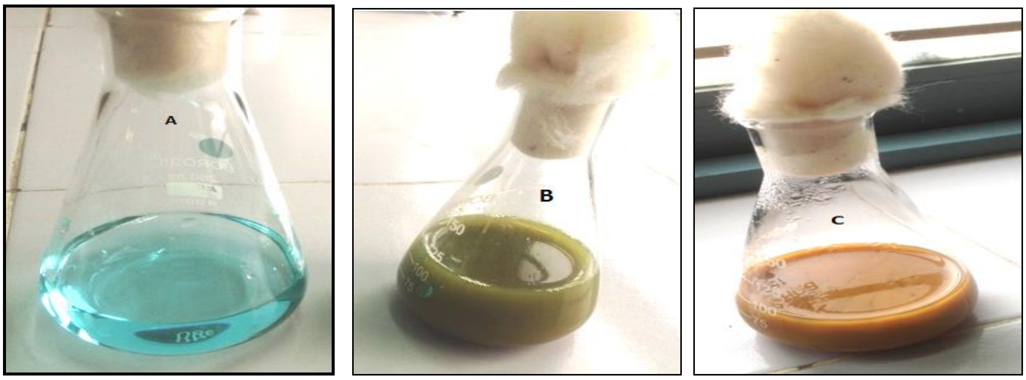
UV-Vis spectroscopy - The measurement of copper nanoparticles synthesized under different conditions like variation of concentration of plant extracts (5 % fruit rind & leaf extract of *Punica granatum*), different molarity of copper sulphate solutions, incubation time, temperature and pH were measured at the particular wavelength of 450 nm as it gave maximum absorption (Syed Thanveer Banu *et al,* 2018).

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used to observe the size, shape of the synthesized nanoparticles. The sample prepared under standardized conditions using 10 mM copper sulphate solution with 5% fruit rind extract was analyzed by SEM and the photographs were obtained.

RESULTS AND DISCUSSION

Influence of various factors on synthesis of copper nanoparticles from different aqueous plant extracts of *Punica granatum* was studied taking into consideration the effect of molarity of copper sulphate solution, incubation period, temperature and pH over a range. A gradual increase in copper nanoparticles synthesis was observed with an increase in the factor under study and decreased when the factor was increased beyond the optimum level.



A-Copper sulphate solution **B-Plant extract and $CuSO_4$** **C-Copper naoparticles**

Figure 1: Color change of green synthesized copper nanoparticles A & B (Pre incubation), C (Post incubation)

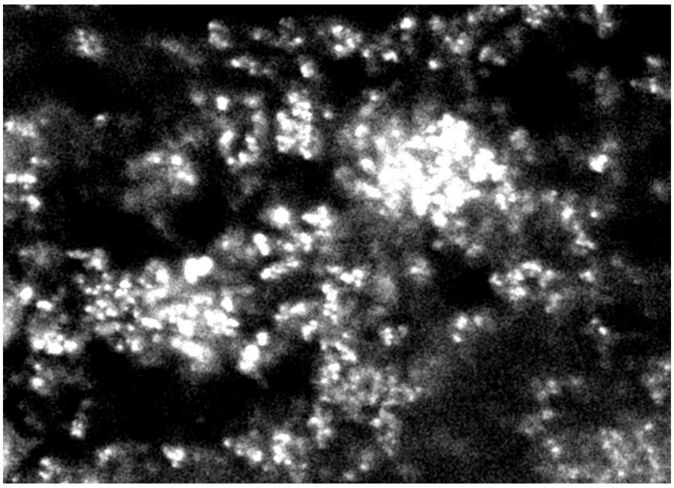


Figure 2: SEM analysis of green synthesized copper nanoparticles

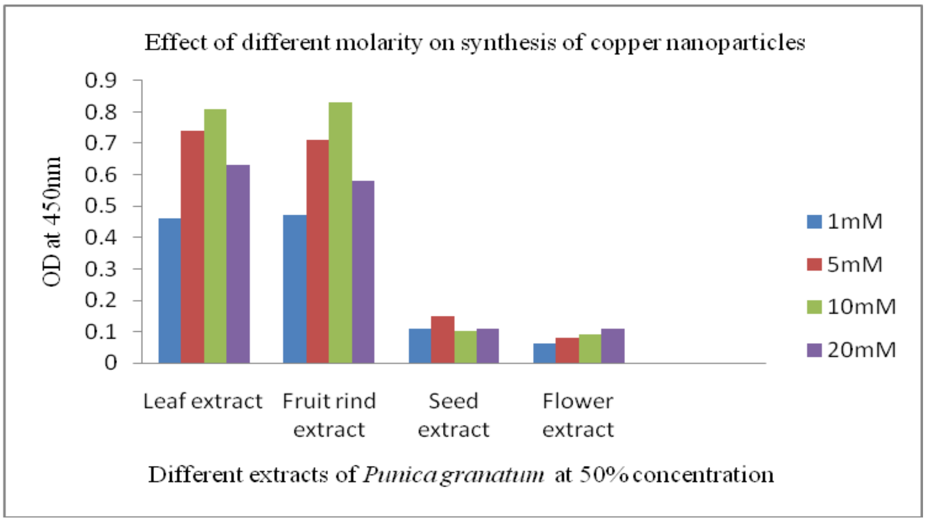


Figure 3: Effect of Molarity on green synthesis of copper nanoparticles with diverse *Punica granatum* plant extract.

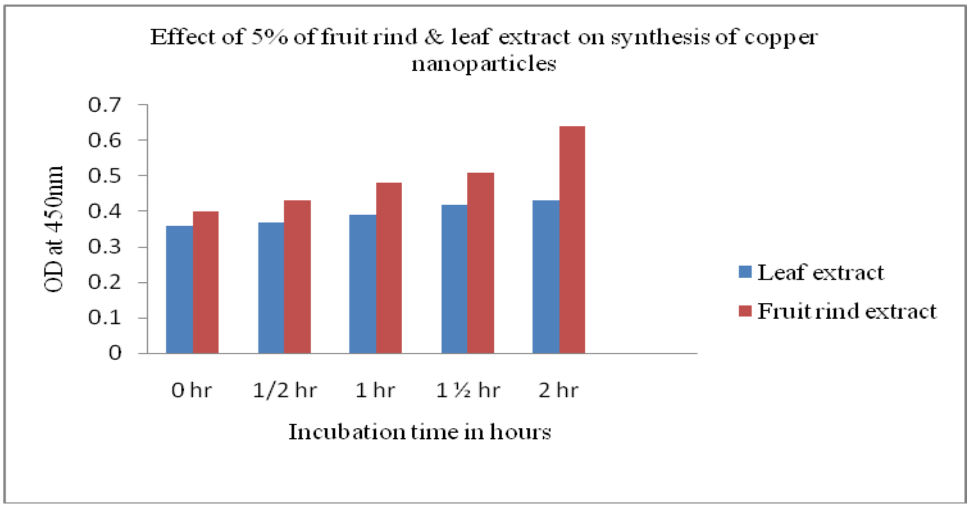


Figure 4: Effect of 5% of fruit rind & Leaf extracts on synthesis of copper nanoparticles

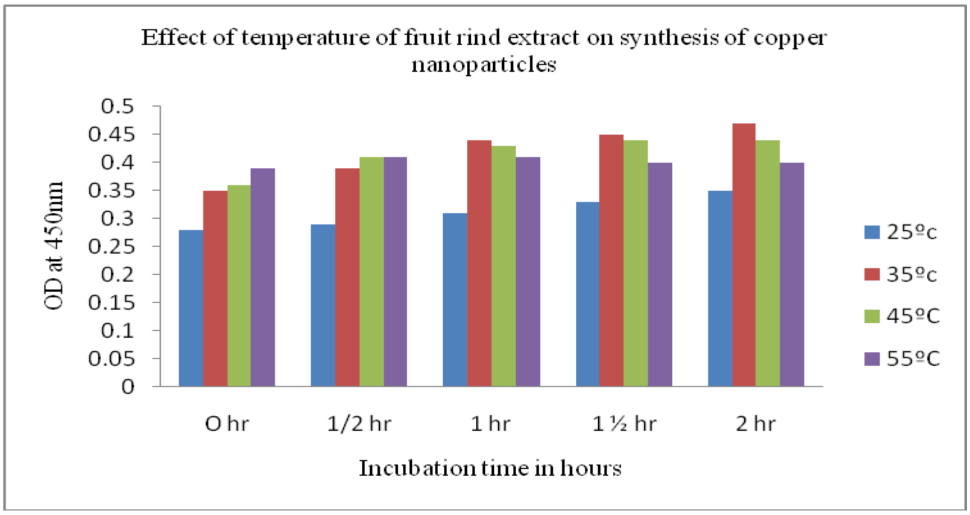


Figure 5: Effect of temperature of fruit rind extract on synthesis of copper nanoparticles

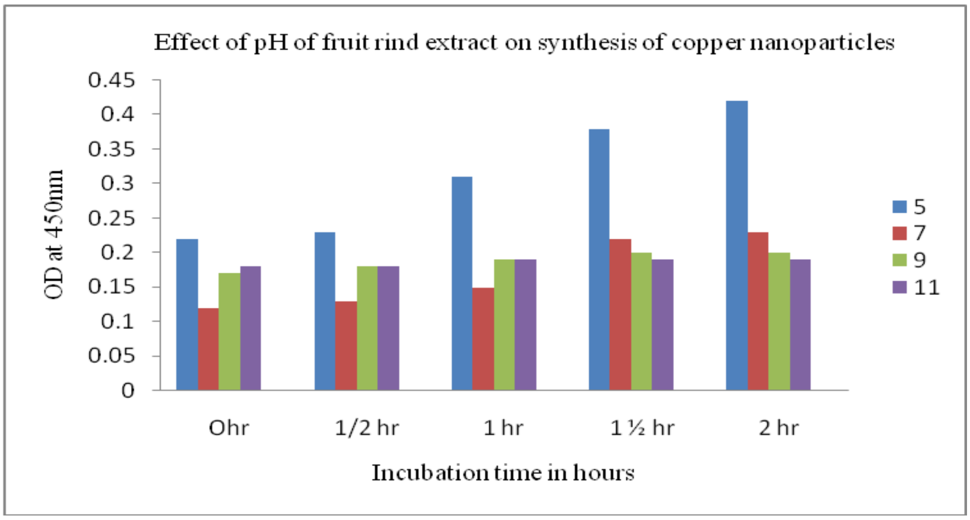


Figure 6: Effect of pH on fruit rind extract on synthesis of copper nanoparticles

The results indicated that the copper sulphate on reaction with plant extract solution was converted to yellow color indicating the formation of copper nanoparticles (Figure 1). SEM images indicate that the nanoparticles prepared from 10 mM copper sulphate with 5% fruit rind extract of *Punica granatum* were spherical in shape (Figure 2). Study of effect of molarity of copper sulphate on different plant extracts of *Punica* *granatum* showed that fruit rind extract and leaf extract showed higher copper nanoparticles with 10 mM copper sulphate solution (Figure 3).

The effective fruit rind extract showed higher copper nanoparticle production when incubated for a period of 2 hours (Figure 4). Similar results were obtained when incubated at a temperature of 35^{0}C (Figure 5) and pH of 5 (Figure 6).

In the present study on factors influencing green synthesis of copper nanoparticles fruit rind extract was found to be effective as it could acts as both reducing and capping agent. Fruit rind is known to be rich in polyphenols and tannins and there are reports of these acting as good reducing and capping agents for metal nanoparticle synthesis (Tufail *et al*., 2013).

Basically, the reduction rate of Cu $^{+2}$ ions increases by increasing the reaction temperature. Therefore the synthesis rate is too high to control particle size at high temperature. These phenomena result in the formation of copper nanoparticles with high averaged size of the copper nanoparticles were precipitated. Thus moderate temperature should be selected for synthesis of the copper nanoparticles with appropriate controlling on size and in the present study 35^0 C was found to be optimum.

It also showed that the pH of the solution has an influence on the progress of bioreduction of copper sulphate solution. The probable kinetic enhancement could be conducive to a reduction in crystallite size because of the enhancement of the nucleation rate (Song *et al,* 2009). Researchers have discovered that pH of the solution medium influences the size and texture of the synthesized nanoparticles. Therefore, nanoparticle size can be controlled by altering the pH of the solution media. The effect of pH on the shape and size of the synthesized nanoparticles was demonstrated by Patra and Kwang-Hyun Baek (Patra *et al* , 2014). The maximum blue shift in SPR peak around the maximum value at pH 5 could be attributing to the decrease in the particle size (Haverkamp *et al*, 2009).

CONCLUSION

The green synthesis of copper nanoparticles using plant extracts is both eco-friendly and cost effective. Plant extracts acts as both reducing and capping agents aiding the formation of more stable nanoparticles thus giving good scope for scale up. The standardized factors for green synthesis of copper nanoparticles are 10 mM copper sulphate solution, 50% concentrated fruit rind extract of *Punica granatum,* incubation time of 2 hours, incubation temperature of 35^{0}C and pH of 5. Thus standardization can facilitate the bulk synthesis of copper nanoparticles commercially and these can be used as antimicrobial agents and help to combat the fast growing drug resistance.

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