International Journal of Applied and Pure Science and Agriculture
ISSN Online - 2394-5532 Impact Factor : 4.446 by SJIF ISSN Print - 2394-823X

Punica granatum flower extract mediated synthesis of Copper oxide Nanoparticle and evolution of its antibacterial activity

Perumal Pon Matheswari[1], Murugan, C[2]. Akila[3], R. Menisha[4], S. Revathi[5],T
[1,3,4,5]*Department of Chemistry, Pope's College (Autonomous)*
[2]*Department of Chemistry, Veerasamy Chettiar College of Engineering and Technology, Puliangudi-627855*

Abstract

Nano copper oxide is extensively used as an important inorganic material in variety of fields such as catalysis, superconductors, ceramicsetc. Owing to its characteristic properties, such as photovoltaic properties, thermal resistance and antioxidant activity it has achieved a huge applications compared with the ordinary copper oxide.In this paper, we report the green synthesis of copper oxide nanoparticle making use of eco-friendlyPunica granatum flower extract. The synthesized CuONPs are characterized by UV-visible absorption spectroscopy,fourier transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD). The UV absorption band at 442 nm confirms the formation of CuO NPs and XRD pattern confirms the crystalline monoclinic structure of CuO NPs. FT-IR reveals the presence of variety of phytochemicals like terpenoids, alkaloids, phenolic along with CuO NPs as capping agents. The synthesized CuO NPs exhibit very good anti bacterial activity of towards both gram positive and negative pathogens.

Keywords- Green synthesis, Punica granatum flower and Copperoxide nanoparticles

I. INTRODUCTION

Nanotechnology plays a vital role in recent years of research[1].It is one of the successful technology finding applications in almost all the fields including pharmaceutical, electronics, bio-medical technological, chemical industry, and cosmetics. In pharmaceuticals, it has been applied to cure infection[2], cancer[3], allergy[4], diabetes[5] and inflammation[6].

Nanoparticle is behaved as a unique because of its large surface area and surface energy. It is highly reactive and can be used, as a good catalyst[7].Metal nanoparticles have been synthesized using variety of methods, which follow either top-down (physical) approach or bottom-up (chemical) approach. Most popular chemical methods produce nanoparticles along with the existence of toxic chemicals on the surfaces, which lead to some adverse effect in medical applications [8]. Environmental friendly and secure technology is the ultimate need for the production of nanoparticles. Green synthesis of NPs making use of plant extracts becomes the ultimate method for the production of metal NPs considering its cost effective, easy scale up and environmental benign steps.

Copper oxide NPs have numerous applications in different fields of research includinghigh-Tc superconductors, gas sensors, optical, electrical, sensors, high magnet resistance materials [9] etc. The reported conventional synthesis of Copper oxide NPs like chemical route [10], hydrothermal method [11], solid-state reaction method [12], thermal decomposition ofprecursor [13],etc leaves some toxic chemicals absorbed at the surface, which can cause adverse outcomes in medical applications.A variety of plant extracts such as *Aloe veraLeaf*[14],*Ixora Coccinea*[15], Pomegranate peel [16], *Magnolia kobus*[17], *Carica Papaya*[18],*Manihot esculenta*[19],*Tridax procumbens*[20], Azadirachta indica [21], *Saraca indica*[22]have been utilized for the greener way production of CuO NPs.

*Punicagranatum (Pomegranate)*belongs to the family of Punicaceae, which is mainly cultivated in India, Spain, America, China and Israel. Various alkaloids, flavonoids, tannins and phenolic compounds[23] extracted from roots, bark, leaves, peel and seed of *Punica granatum* is found to cure heart disease, anemia and some type of cancers[24]. The flower contains Gallic acid, ursolic acid [25], triterpenoids andasiatic acid. No study has been found in the literature for the synthesis of CuO NPs using *Punica granatum* flower extract. Therefore, we report here, the synthesis and characterization CuO NPs using *Punica granatum* flower extract. We also report the anti bacterial activity of the synthesized CuO NPs.

II. MATERIALS AND METHODS

2.1 Materials

The *Punicagranatum* flower samples were collected from sawyerpuram, Tuticorin district, TamilNadu, India. Copper chloride ($CuCl_2.2H_2O$) and Sodium hydroxide were purchased from Standard Scientific Supplies chemicals (P) Ltd,Tirunelveli, India.

2.2 Methods

2.2.1 Preparation of plant Extract

10 g of the collected flower samples was washed thoroughly with distilled water,mixed with 100 ml distilled water and boiled for 15-20 minutes. The extract was filtered through whatmann no. 1 filter paper and stored in a air tight container under 4^oC for further use.

2.2.2 Green-Synthesis of Copper oxide Nanoparticles

40 ml 4mM copper chloride aqueous was treated with 10 ml flower extract and the mixture wasstirred at room temperature (at 2000 rpm). The stirring was continued for till to geta dark yellow color from thelight straw yellow color. Then the mixture was heated at 80^oC for 2 minutes and treated with 1M sodium hydroxide drop by drop. The reddish brown precipitate appeared indicating the formation of water soluble monodispersed copper oxide nanoparticles. The concentration of copper chloride was varied from 1 to 4 mM solution. The reddish brown precipitate was then taken out and washed repeatedly with deionized water to remove the impurities to get the final product.

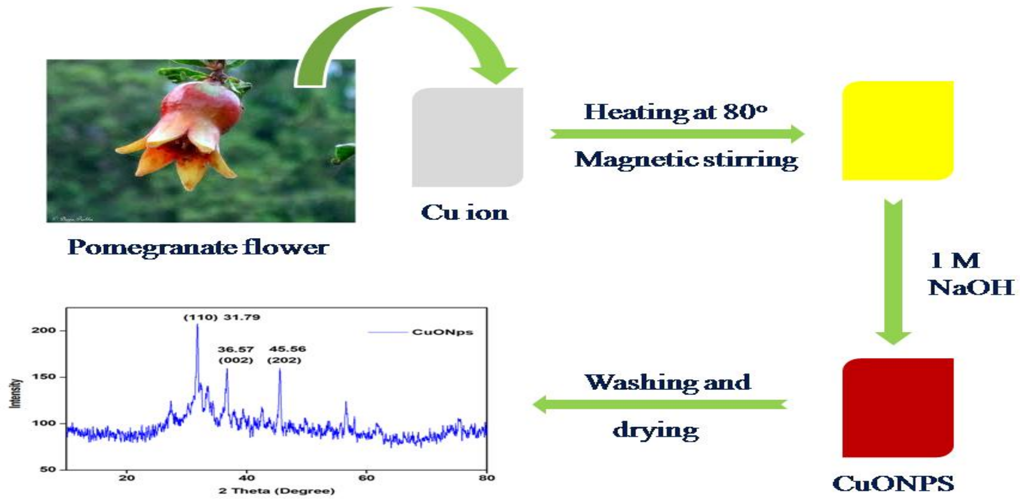

Figure .1 Formation of Copper oxide nanoparticle

2.3Characterization of copper oxide Nanoparticles

The green synthesized CuO NPs was analyzed usingUV-visible, FT-IR and XRD analyses. Jasco V-550 spectrophotometer at the range of 200-800nm was used to get absorption maximum.CuO NPs were centrifuged at 9500 rpm for 20 min, dried in a hot air oven and ground to get powder. FT-IR spectrumof the powdered sample wasrecorded with Jasco 5300 model FTIR instrument using KBr pellet at the spectral range of 400-4000 cm^{-1}. The xrd pattern of the air dried CuONPs wasanalyzed by PAN analytical X'PERT-PRO powder X-ray diffractometer (XRD) using

Volume 04, Issue 1, [January- 2018] ***e-ISSN: 2394-5532, p-ISSN: 2394-823X***

Cu-K_α radiation (1.5406Å) in the 2θ range 20-80°. The anti-bacterial activity synthesized CuONPs was carried out using gel-diffusion method.

III. RESULT AND DISCUSSION

3.1 Visual Characterization

The formation of CuO NPs via a greener route using *Punica granatum* flower extract was well visualized from the Figure 2. The distinct color of the flower extract (A in Fig. 2) has acquired a reddish brown color upon addition of $CuCl_2$and NaOH. The color changes indicate the formation of CuONPs.

Figure .2 Visual observations (A) Plant extract (B) Copper chloride solution and (C) formation of CuO NPs

3.2 UV-Visible analysis

The bio synthesis for the formation of copper oxide nanoparticles using *Punica granatum flower* extract was reported. Formation of eco-friendly copper oxide nanoparticles had been showed via UV-VIS spectrometry. Figure.3 indicates the UV–Vis absorption spectrum of copper oxide nanoparticles and *Punica grantum* flower extract. The synthesized CuO NPs showed an absorption maximum at 442 nm due to surface plasmon resonance of CuO NPs[26].

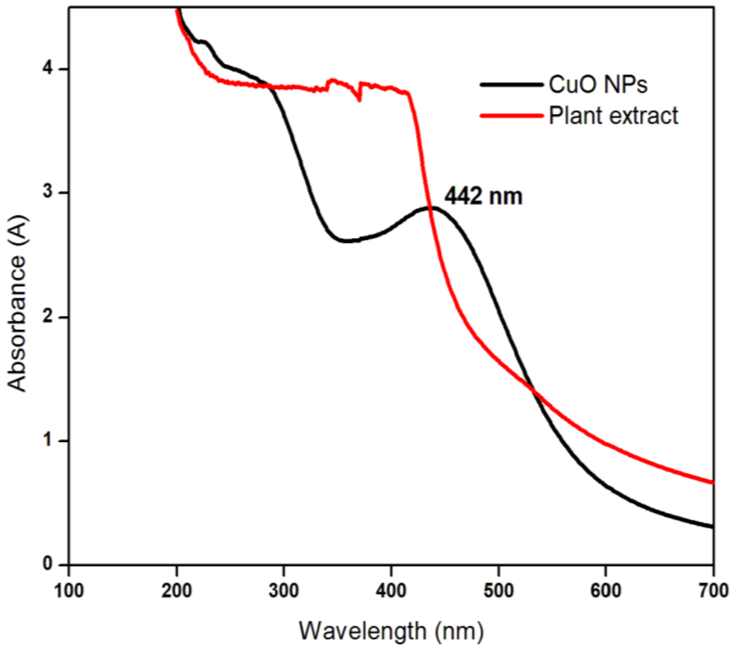

Figure .3 UV-Vis absorption spectrum of green synthesized CuONPs and *Punica granatum* flower extract

3.3 FT-IR Analysis

FT-IR spectroscopy was used to investigate the interactions between different species and changes in chemical compositions of the mixtures. FT-IR measurements of both the aqueous *Punica granatum* flower extract and the air-dried CuO NPs were carried out to identify the possible bio-molecules responsible for the reduction, capping and efficient stabilization of the bio-reduced CuONPs. The FT-IR spectra of the *Punica granatum* flower extract and the synthesized CuONPs are shown in Figure 4. The *Punica granatum* flower extract displays a number of adsorption peaks, reflecting its complex nature. The presence of terpenoids in *Punica granatum* flowerextract can be identified by the strongest peaks of hydroxyl at 3396 cm^{-1}, α, β- unsaturated ketone band at 1716 $cm^{-1}$and olefinic band at 1617cm^{-1}. Primary and secondary alcohols showed bands at 1043 cm^{-1} as well as the peaks around 1447 cm^{-1} attribute to aliphatic C-H bending modes.
The major peak was observed in CuONPs to be 546 $cm^{-1}$was due to stretching of Cu-O bond [26] . In addition the disappearance of $\gamma_{C=O}$ stretching vibration of the α,β- unsaturated ketone at 1716 cm^{-1} confirm that the reduction and the stabilization of CuO NPs.

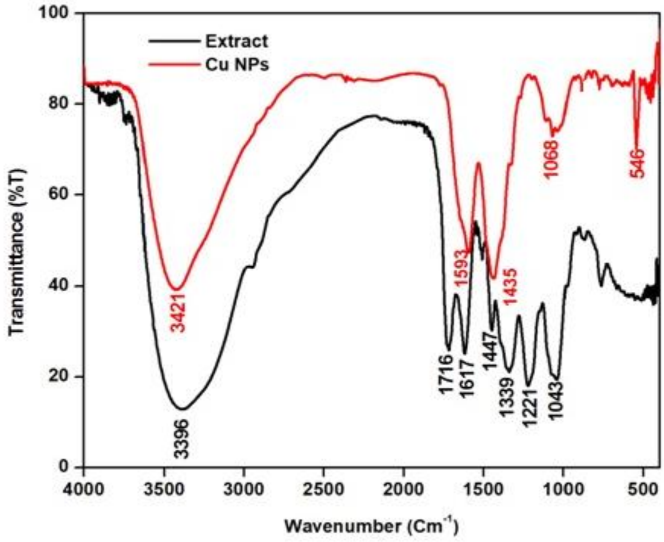

Figure .4 FT-IR spectra of flower extract and CuO NPs

3.4 X-Ray Diffraction Analysis

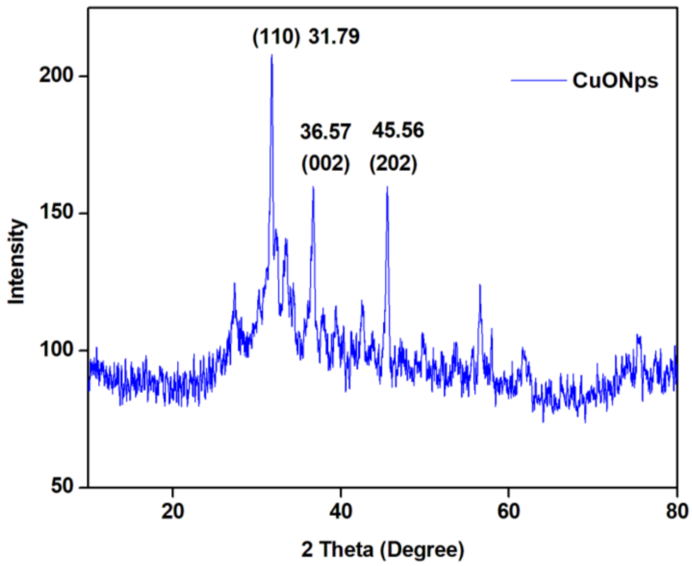

Figure.5 XRD pattern of the green synthesized CuONPs

Figure 5shows the X-ray diffraction (XRD) pattern of the CuONPs synthesized using*Punica granatum* flower extract. The XRD pattern revealed the orientation and crystalline nature of CuO NPs. The peak position with 2θ values of 31.79,36.57and 45.56 with respective planes 110, 002 and 202 confirmed the formation of monoclinic crystalline structure of CuO NPs (JCPDS-80-1268)[27]. The average crystallite size of the synthesized copper oxide nanoparticles was calculated using Debye-Scherrer equation. The analysis of 001 plane revealed that the average crystallite size of the CuO NPS as 30.87 nm.

3.5 Anti-bacterial activity

Anti-bacterial activity of synthesized CuO NPs wasstudied against gram positive *Staphylococcus aureus*, gram negative*Escherichia coli* and *Pseudomonas aeruginos* by using gel-diffusion method.*Punica granatum*flower extract was used as blank and the activity of the sample was compared to that of the standard drug Amikacin.

The results showed that the synthesized CuO NPs were active against gram negative species E.coli and P.*aeruginos* with the inhibition zone of diameter 8, 11mm respectively. However, CuO NPs has only very feebleactivity towards gram positive species S.aureus.

Figure.6Zone of inhibition bacteria E-Coli, P.*aeruginos* and S.aureus

IV. CONCLUSION

Copper oxide nanoparticles are synthesized via eco-friendly greener route. *Punica granatum* flowerextract successfully involved in the preparation of CuO NPs. The extract functions as both reducing and stabilization agent for CuO NPs. FT-IR spectral analysis shows the presence of terpenoids in *Punica granatum* flowerextract which could involve in a dual role of reduction and stabilization of CuO NPs. The average crystallite size of the synthesized CuO NPs is 30.87. The synthesized CuONPs are highly stable and have significant effect on both gram negative E.coli and P.*aeruginos* bacteria.

BIBLIOGRAPHY

[1] Vasudev, D. Kulkarni, Pramod, S. Kulkarni.2013. Green Synthesis of Copper Nanoparticles Using Ocimum Sanctum Leaf Extract *InternationalJ. Chemical Studies*, **1(3):**1-4.

[2] Furno, F. Morley, KS. Wong, B. Sharp, BL. Arnold, PL. Howdle, SM. 2004. Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection. *J Antimicrob Chemother*., **54**: 1019–1024.

[3] Brigger I, Dubernet C, Couvreur P. 2012. Nanoparticles in cancer therapy and diagnosis. *Adv Drug Deliv Rev*., **64**: 24–36.

Volume 04, Issue 1, [January- 2018] e-ISSN: 2394-5532, p-ISSN: 2394-823X

[4] Roy, K. Mao, HQ. Huang, SK. Leong, KW. 1999. Oral gene delivery with chitosan-DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. *Nat. Med*. **5**: 387–391.

[5] Basarkar, A. Singh, J. Poly 2009 (lactide-co-glycolide)-polymethacrylate nanoparticles for intramuscular delivery of plasmid encoding interleukin-10 to prevent autoimmune diabetes in mice. *Pharm Res*., **26**: 72–81.

[6] Wilson, DS. Dalmasso, G. Wang, L. Sitaraman, SV. Merlin, D. Murthy, N. 2010. Orally delivered thioketal nanoparticles loaded with TNF-α–siRNA target inflammation and inhibit gene expression in the intestines. *Nat. Mater.*,**9**: 923–928.

[7] Divya C, Muthuvinothini A. 2015. Nanoscience and Nanotechnology. Edorium J Nanotechnol,**2** :1–3.

[8] R. M. Jose, L. E. Jose, C. 2005. The bactericidal effect of silver nanoparticles. Alejandra Nanotechnology **16,** 2346–2353.

[9] Naika, H.R. Lingaraju, K. Manjunath, K. Kumar, D. Nagaraju, G. Suresh, D. Nagabhushana, H. 2015. Green synthesis of CuO nanoparticles using Gloriosasuperba L.extract and their antibacterial activity. *J. TaibahUniv Sci.*, **9**: 7-12.

[10] Jyoti Mayekar, Vijay Dhar, Radha, S. 2014. Synthesis of Copper Oxide Nanoparticles Using Simple Chemical Route. *International Journal of Scientific & Engineering Research.*, **5**(10): 928-930.

[11] Roble, M and Diaz-Droguett, D. 2017. Effect of O_2 pressure on the hydrothermal growth of CuO hierarchical microstructures: characterization and hydrogen adsorption capacity. *Mater. Res. Exp.*,**4**(8): 085502

[12] Xu, J.F. Ji, W. Shen, Z.H. Tang, S.H. Ye, X.R. Jia, D.Z. X.Q. Xin,J. 2000. Preparation and characterization of CuONanocrystals. *Solid state chem*., **147(2)**: 516–519.

[13] Xu, C.K. Liu, Y.K. Xu, G.D. Wang, G.H. 2002. Preparation and characterization of CuO nanorods by thermal decomposition of CuC_2O_4 precursor. *Mater. Res. Bull*., **37(14):** 2365–2372.

[14] Vijay Kumar, P.P.N. Shameem, U. Pratap Kollu, Kalyani R.L. Pammi, S.V.N. 2015. Green Synthesis of Copper Oxide Nanoparticles Using Aloe vera Leaf Extract and Its Antibacterial Activity Against Fish Bacterial Pathogens. *BioNanoSci.*, **5(2):** 65-122.

[15] Yedurkar1, S.M. Maurya, C.B. Mahanwar, P.A. 2017. A Biological Approach for the Synthesis of Copper Oxide Nanoparticles by Ixora Coccinea Leaf Extract. *J.Material and Environmental Science.*,**8(4)**: 1173-1178.

[16] Alaa Y. Ghidan, Tawfiq M. Al-Antary, Akl M. Awwad. 2016. Green synthesis of copper oxide nanoparticles using Punica granatum peels extract: Effect on green peach Aphid. *Environmental Nanotechnology Monitoring & Management*., **6**: 95–98.

[17] Lee, H. J. Lee, G. Jang, N. R. Yun, J. H. Song, J. Y. Kim, B. S. 2011. Biological synthesis of copper nanoparticles using plant extract. *Nanotechnology.*,**1(1)**: 371-374.

[18] Sankar, R. Manikandan, P. Malarvizhi, V. Fathima, T. Shivashangari, K. S. Ravikumar, V. 2014. Green synthesis of colloidal copper oxide nanoparticles using Carica papaya and its application in photocatalytic dye degradation. *Spectrochim. Acta Mol. Biomol. Spectrosc*., **121**: 746-750

[19] Ramesh, C. Hari Prasad, M. Ragunathan, V. 2011. Antibacterial Behaviour of Cu_2O Nanoparticles Against Escherichia coli; Reactivity of Fehling's Solution on Manihotesculenta Leaf Extract. *Curr. Nanosci.*,**7(5)**: 770-775.

[20] Gopalakrishnan, K. Ramesh, C. Ragunathan, V. Thamilselvan, M. 2012. Antibacterial activity of copper oxide nanoparticle on E coli synthesized from *TridaxProcumbens* leaf extract and surface coating with polyaniline. *Dig. J. Nanomater. Biostruc.*,**7**: 833-839.

[21] Ansilin, S. Kavya Nair,C. Aswathy, V. Rama, J. Peter,J. Jeyachynthaya, P. 2016. Green synthesis and charterization of copper oxide nanoparticles using Azadirachta india (Neem) Leaf Aqueous Extract. *J. Nanoscience and technology*.,**2(5):** 221-223**.**

[22] Kollur Shiva, P. Alakananda.P. Govindaraju,S. and Shivamallu, C. 2017. Aqueous Extract of Saraca indica Leaves in the Synthesis of Copper Oxide Nanoparticles: Finding a Way towards Going Green. *J. Nanotechnology*,2017:1-6

[23] Edeoga, H.O. Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.*,**4(7):** 685–688.

[24] Taruna and Jyotsna kaushal. 2017. Green Synthesis of Silver Nanoparticles by Using Pomegranate and Their Antimicrobial Activity-A Revolution. *J. Chemistry, Environmental Sciences and its Applications*., **1(1):** 1–10.

[25] Huang, T.H. Yang, Q. and Harada, M. 2005. Pomegranate Flower Extract Diminishes Cardiac Fibrosis in Zucker Diabetic Fatty Rats: Modulation of Cardiac Endothelin-1 and Nuclear Factor-kappaB Pathways, *J. Cardiovasc Pharmcol*, **46(6):** 856–862.

[26] Syame, W.S. Mahmed, S. M. Mohamoud, R. K. Omara, S. T. 2017. Synthesis of Copper-Chitosan Nanocomposites and its Application in Treatment of Local Pathogenic Isolates Bacteria. Orient *J. Chem*,**33(6).**

[27] Kankanit Phiwdang ,Sineenart Suphanki Wanichaya Mekprasart and Wisanu Pecharap. 2013. Synthesis of CuO Nanoparticles by Precipitation Method Using Different Precursors. *Energy Procedia.*, **34**: 740 – 745.

