International Journal for Research in Engineering Application & Management (IJREAM)
ISSN : 2454-9150 Vol-04, Issue-09, Dec 2018

Biosynthesis, Characterization And Antibacterial Activity Of Copper Oxide Nanoparticle Using *Murraya Koenigii* (Curry Leaf)

T. Deepika[1] and C.M. Noorjahan*[2]

[1]Ph.D. Research Scholar, P.G. & Research Department of Zoology, JBAS College for Women (Autonomous), Teynampet, Chennai, Tamil Nadu, India. tdeepika15@gmail.com

[*2] Assistant Professor, P.G. & Research Department of Zoology, JBAS College for Women (Autonomous), Teynampet, Chennai, Tamil Nadu, India. cmnoorjahan@gmail.com

Abstract - The present study was aimed to biosynthesize the copper oxide nanoparticle using aqueous leaves extract of *Murraya koenigii* (Curry leaf). UV-Vis spectroscopy and SEM analysis were carried out to identify the nanoparticle. The antibacterial activity of biosynthesized copper oxide nanoparticle was investigated using Agar well diffusion method. The results of the UV-Vis Spectroscopy showed that the maximum peak of copper oxide nanoparticle was confirmed at 410 nm. SEM images was carried out to determine the morphology of the nanoparticle. The shape of the nanoparticle was spherical and spindle. Further, the study was extended to determine the antibacterial activity of copper oxide nanoparticle, which showed significant inhibitory activity in *Salmonella typhii* than the other bacterial species.

Keywords: Antibacterial activity, Characterization, Copper Oxide nanoparticle, Curry leaf, Murraya koenigii.

I. INTRODUCTION

Nanotechnology plays an important role in the modern research [1]. Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions controlled dispersity and their potential use for human benefits [2-3]. This technology can be applicable in a wide range of fields such as pharmacology, food and nutrition, chemical industries, energy sciences, cosmetics, further, it can be used for the treatment of infections, cancer, allergies and diabetes [4-6]. Synthesis of metal nanoparticles has gained much importance because their physical-chemical properties differ from those of the bulk particles [7]. The convergence of metallic nanoparticles and biological techniques has created a new field of nanomedicine [8]. The metal nanoparticles are finding applications in optoelectronics, nanodevices, nanoelectronics, nanosensors, information storage and catalysis. Metallic nanoparticles are of great interest due to their excellent chemical, physical, medicinal and catalytic properties [9]. Among various metal particles, copper nanoparticles have attracted more attention because of their catalytic, optical, electrical and antifungal/antibacterial applications [10-11]. It is evident from the literature that, synthesis of stable Copper nanoparticle (CuNP) is the challenging task and various methods have been developed to synthesize Copper nanoparticles including physical, chemical and biological processes [12].

Many researchers are involved in detecting new technologies and effective antibacterial agents which led to the use of Ag or Cu based antiseptics that may be linked to the broad spectrum of activity and less susceptibility of developing bacterial resistance than antibiotics alone [13].

Green chemistry refers to the design and development of chemical products and processes in order to minimize hazards on the environment [14]. Green synthesis has numerous advantages compared to other methods, including cost-effectiveness, simple, use of lower temperatures, use of non-toxic materials, as well as compatibility with applied medical and nutritional programs [15]. Green synthesis method is being developed and is an environmentally-friendly method as well [16]. CuNP synthesis has attracted particular interest, compared with other NP's as their useful properties are achievable at costs lower than Silver and Gold [17]. Copper nanoparticle (CuNP's) have been used in various fields including agricultural, industrial engineering and technological fields [18]. In recent years, Biosynthesis of copper oxide nanoparticles was achieved by using micro-organisms [19], Plant extract [20], seed [21] and bark [22]. Copper and CuNP's have been synthesized by a variety of plant extracts [9]. The nanoparticles synthesized with plant extracts were found to exert biological activities like antibacterial, antioxidant and anticancer [23-25]. The plant-

 DOI : 10.18231/2454-9150.2018.1220

mediated synthesis is a rapid, flexible, and suitable process for large scale production of nanoparticles [26].

Thus the main aim and objectives of the study is to synthesize Copper oxide nanoparticle using aqueous leaves extract of *Murraya koenigii* (Curry leaf) and to determine its Characterization and antimicrobial activities.

II. EXPERIMENTAL DETAILS

Murraya koenigii (Curry Leaf) leaves were collected from the local market and brought to the laboratory. The leaves were thoroughly washed with normal water and then with distilled water and air dried for about a week in room temperature. Then it is blended into fine powder. Aqueous extract of *Murraya koenigii* (Curry leaf) leaves were carried out by following the procedure [27]. 5 grams of *Murraya koenigii* (Curry leaf) leaves was dissolved in 100ml of distilled water for 20 minutes and boiled at 50°C. The leaves extract was removed from heat and allowed to cool. After cooling the extract were filtered using Whatmann No.1 filter paper and stored at 4°C for further use. Biosynthesis of Copper oxide nanoparticle was carried out by following the procedure [27]. 80 ml of 1mM of Copper sulphate was dissolved in 20 ml of already prepared *Murraya koenigii* (Curry leaf) leaves extract and mixed thoroughly. The reduction of copper ions was confirmed by the change in colour of the reacted solution. The reacted solution was further dried at 60°C overnight and calcined at 100°C for one hour. The resultant powdered sample was subjected for the characterization. The green synthesized nanoparticle was subjected for the analysis of UV-Spectroscopy analysis using Labtronic digital spectrophotometer. The size range of the Spectrophotometer is 300-700 nm. Scanning Electron Microscopy was used to study the morphology of biosynthesized Copper oxide nanoparticle. The SEM analysis was performed at an accelerating voltage of 20 kV. Further, antibacterial activity of biosynthesized Copper oxide nanoparticle against four isolates of bacteria namely, *Escherichia coli*, *Staphylococcus aureus*, *Psedumonas aeroginosa* and *Salmonella typhii* were carried out by agar well diffusion method by following the Standard methods [28].

III. RESULTS AND DISCUSSION

Metals have been used for centuries as antimicrobial agents. Silver, Copper, Gold, Titanium, Zinc have gained particular attention, each having different properties and spectra of action [29]. With respect to nanoparticles, the antimicrobial properties of silver and copper have received the most attention. However, copper is cheaper than silver, readily miscible with polymers and relatively stable chemically and physically [30]. Much work has been done in bioreduction of metal nanoparticles by a combination of biomolecules found in plant extracts (enzymes, proteins, aminoacids, vitamins, polysaccharides and organic acids such as citrates) and the respective role of phytochemicals [31-33].

3.1. SYNTHESIS OF COPPER OXIDE NANOPARTICLE USING *MURRAYA KOENIGII* (CURRY LEAF) LEAVES

The results of synthesis of Copper oxide nanoparticle using *Murraya koenigii* (Curry leaf) showed the colour change of the reactant. During the exposure of leaves extract to Copper sulphate solution, reduction of Copper ions into Copper oxide nanoparticle is visualized as a result of change in colour from dark green to pale green colour, which occurred due to the Surface Plasmon Resonance phenomenon (Plate-1). The metal nanoparticles have free electrons, which helps in the formation of the Surface Plasmon Resonance absorption band.

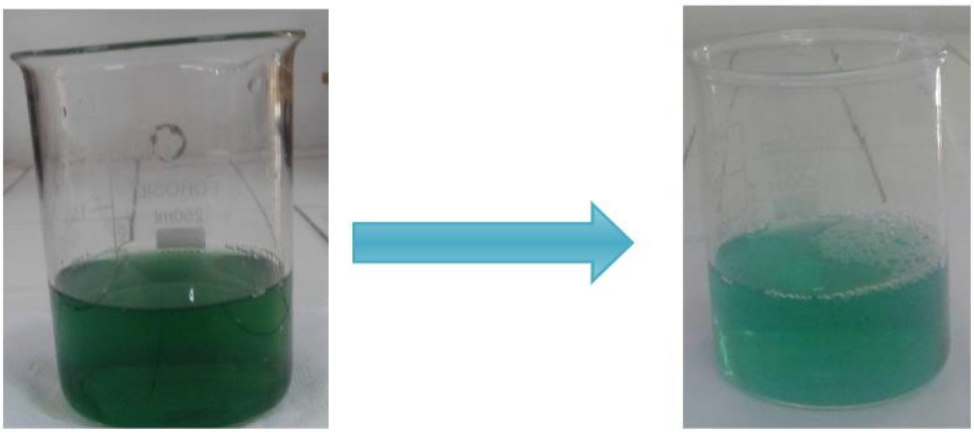


Plate – 1 : Reduction of Copper ions into Copper oxide nanoparticle

3.2. CHARACTERIZATION OF BIOSYNTHESIZED COPPER OXIDE NANOPARTICLE USING *MURRAYA KOENIGII* LEAVES

3.2.1. UV- VISIBLE SPECTROSCOPY

The results of UV visible spectroscopy analysis of biosynthesized Copper oxide nanoparticle from *Murraya koenigii* (Curry leaf) leaves was represented in Fig 1. Absorption spectra of the reduction of copper ions was

 DOI : 10.18231/2454-9150.2018.1220

formed in the reaction media which has the absorbance's peak at 410 nm.

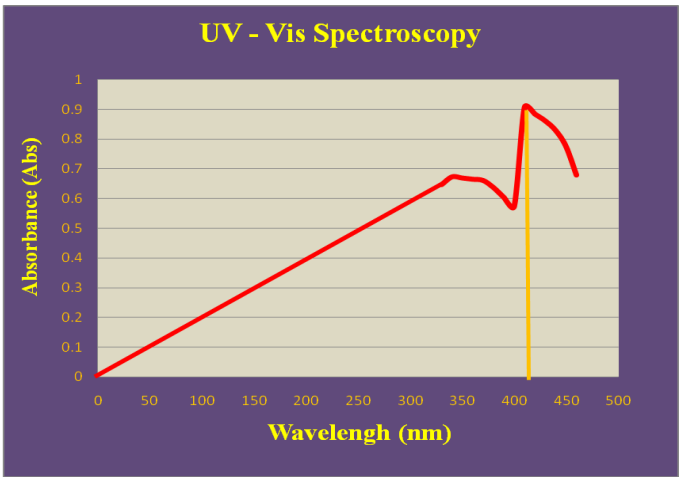


Figure – 1 : UV-Visible Spectroscopy of Biosynthesized Copper Oxide nanoparticle

3.2.2. SCANNING ELECTRON MICROSCOPE (SEM)

The result of Scanning Electron Microscope studies of biosynthesized Copper oxide nanoparticle using Curry leaves (*Murraya koenigii*) was represented in Fig - 2. The results of the study revealed that the diameter of the Copper oxide nanoparticle synthesized using *Murraya koenigii* leaves extract ranged from 49-118 nm in diameter and the shape was found to be spherical and agglomerates were also observed. Similar results were observed which showed that the copper nanoparticle produced by *Penicillium citrinum* was spherical shaped [34].

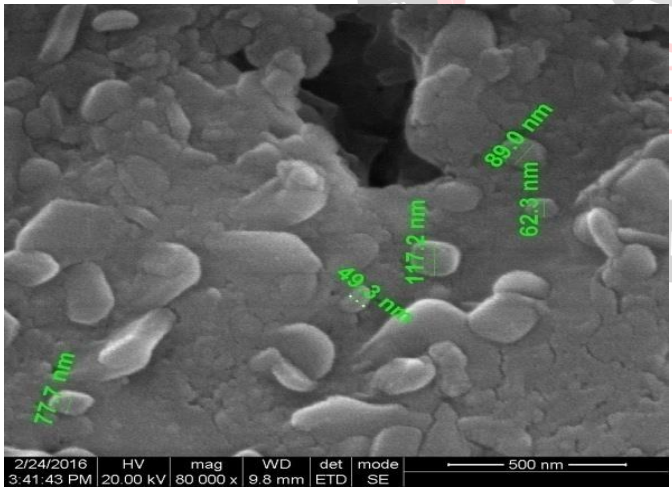


Figure – 2 : Scanning Electron Microscope (SEM) of Biosynthesized Copper Oxide nanoparticle

3.3. ANTIBACTERIAL ACTIVITY OF BIOSYNTHESIZED COPPER OXIDE NANOPARTICLE

The result of the antibacterial analysis was determined by the formation of zone of inhibition for four bacterial species namely, *Escherichia coli*, *Staphylococcus aureus*, *Psedumonas aeroginosa* and *Salmonella typhii*.

The growth of the bacterial samples were analyzed against biosynthesized Copper oxide nanoparticle after 24 hours of incubation at 37°C. The inhibition for the antibiotics was found in four bacterial samples. The samples were loaded with different concentrations of biosynthesized copper oxide nanoparticle such as 1000 µg, 500 µg, 250 µg and 125 µg along with 10 µg of positive control (Streptomycin antibiotic) and negative control (DMSO) were depicted in fig - 3.

The results of antibacterial activity of biosynthesized copper oxide nanoparticle were expressed in Mean±St. Dev. (Table-1). *Escherichia coli* was found to have 24.66±0.577 mm, 11.66±1.154 mm and 8.66±1.527 mm of zone of inhibition in positive control, 1000 µg and 500 µg concentrations. *Staphylococcus aureus* was found to have 22.66±0.577 mm, 26.33±0.577 mm and 9.33±1.154 mm of zone of inhibition in positive control, 1000 µg and 500 µg concentrations. *Pseudomonas aeruginosa* was found to have 25±1.732 mm, 15.66±0.577 mm, 11.66±1.527 mm and 9.66±0.1527 mm of zone of inhibition in positive control, 1000 µg, 500 µg and 250 µg concentrations. *Salmonella typhii* was found to have 36.66±2.08 mm, 20.33±1.52 mm, 15.33±0.57 mm, 14.33±1.52 mm and 12±1.73 mm of zone of inhibition in positive control, 1000 µg, 500 µg, 250 µg and 125 µg concentrations.

Name of the organisms	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhii*	*Staphylococcus aureus*
Positive Control	24.66±0.577	25±1.732	36.66±2.081	22.66±0.577
Negative Control	-	-	-	-
1000	11.66±1.154	15.66±0.577	20.33±1.52	26.33±0.577
500	8.66±1.527	11.66±1.527	15.33±0.577	9.33±1.154
250	-	9.66±1.527	14.33±1.527	-
125	-	-	12±1.732	-

'-' – Absence of formation of Zone of inhibition ; ±Standard Deviation

Table – 1 : Antimicrobial activity of Biosynthesized Copper oxide nanoparticle

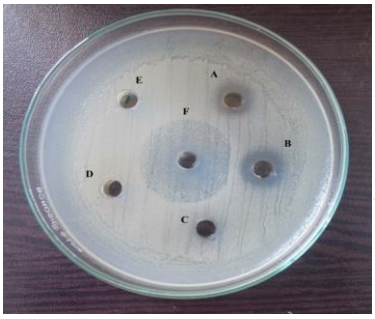
 DOI : 10.18231/2454-9150.2018.1220

Bacterial susceptibility to nanoparticles depends on several factors such as the bacterial strain, the type and size of nanoparticles, the nature of the initial growth media and cell concentration. In general, Gram negative organisms are more resistant to the ionic effect of copper nanoparticles, than Gram-positive bacteria [35]. There is a growing interest in the application of nanoparticles as oral antimicrobials to control various infections, for their biocidal properties and anti-adhesive capabilities against bioflims [30].

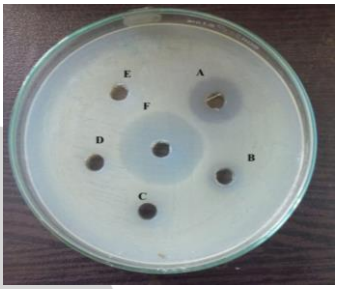
Copper used as an antimicrobial agent for decades has revealed a strong antibacterial activity. Copper nanoparticles are known to exhibit wide range of antibacterial activity against different strains of gram positive and gram negative bacteria [31]. Antibacterial activity can be attributed to disruption of cell membrane due to the release of copper ions from CuO Np's, which attack to negatively charged bacterial cell wall and rupture it, thereby causing protein denaturation and cell death [32].

Thus by comparing the antibacterial activity of all the four bacterial species, biosynthesized copper oxide nanoparticle showed the significant zone of inhibitory activity against *Salmonella typhii* than the other bacterial species.

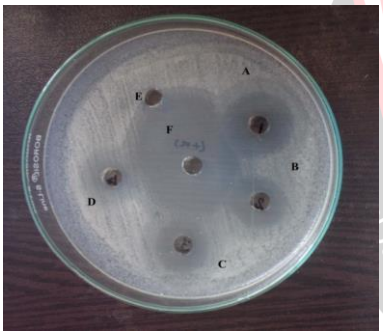
A. *E. coli*



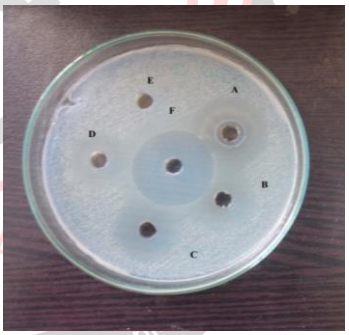
B. ***Stapylococcus aureus***



C. ***Pseudomonas aeruginosa***



D. *Salmonella typhii*



A - 1000 µg ; B - 500 µg ; C - 250 µg ; D - 125 µg ; E - -ve control (DMSO) ; F - +ve control (Streptomycin)

Figure – 3 : Antibacterial activity of biosynthesized Copper oxide nanoparticle

IV. CONCLUSION

The field of nanotechnology is the development of reliable and eco-friendly processes for the synthesis of metal nanoparticles. Biosynthesized copper oxide nanoparticles using plants leaves extract acts as eco-friendly path of synthesis which can be adopted for the bulk production of CuNP's. The results confirmed the reduction of copper sulphate to copper oxide nanoparticle using UV-Vis spectrophotometer analysis and the morphology of the biosynthesized nanoparticle using SEM analysis showed spherical and spindle shaped nanoparticle. Further, antibacterial activity of biosynthesized copper oxide nanoparticle was determined by agar well diffusion method. Thus from the results of the above investigation, it can be concluded that the biosynthesized copper oxide nanoparticle using green plants extracts is a very cost effective, safe, non-toxic, eco-friendly route of synthesis which can be manufactured at a larger scale. Synthesized Copper oxide nanoparticle showed significant antibacterial activity which can be implemented as alternate sources in various medical applications.

REFERENCE

[1] F. Furno, K.S. Morley, B. Wong, B.L. Sharp, P.L. Arnold, S.M. Howdle, R. Bayston, P.D. Brown, P.D. Winship, and H.J. Reid, "Silver nanoparticles and poly meric medical devices : A new approach to prevention of infection," *Journal of Anitmicrob. Chemother*, vol. 54, pp. 1019-1024, Dec. 2004.

[2] Abhishek Kaler, Navin Patel, and Uttam Chand Banerjee, "Green synthesis of silver nanoparticles," *Current Research and Information on Pharmaceutical Sciences*, vol. 11, no. 4, pp. 68-71, 2010.

 DOI : 10.18231/2454-9150.2018.1220

[3] I. Brigger, C. Dubernet, and P. Couvreur, "Nanoparticles in Cancer therapy and diagnosis," *Adv. Drug Deliv. Rev.*, vol. 64, pp. 24-36, Dec. 2012.

[4] K. Roy, H.Q. Mao, S.K. Huang, and K.W. Leong. "Oral gene delivery with chitosan DNA nanoparticles generates immunological production in murine model of peanut allergy," *Nat. Med.*, vol. 5, pp. 387-391, May 1999.

[5] A. Basarkar, and J. Singh, "Poly (Lactide-co-glycolide)-polymethacrylate nanoparticles for intramuscular delivery of plasmid encoding interleukin-10 to prevent auto-immune diabetes in mice," *Pharm. Res.*, vol. 26, pp. 72-81, Jan. 2009.

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

[7] M. Jayandran, M. Muhamed Haneefa, and V. Balasubramanian, "Green synthesis of Copper nanoparticles using natural reducer and stabilizer and an evaluation of antimicrobial activity," *Journal of Chemical and Pharmaceutical Research*, vol. 7, no. 2, pp. 251-259, 2015.

[8] D.S. Goodsell, "Bionanomedicine in action. In : Goodsell D.S. editor. Bionanotechnology : Lessons from nature," Hoboken, NJ., John Wiley and Son's Inc., 2004.

[9] T.T. Lee, E. Fribourg-Blanc, and M.C. Dang. "Synthesis and optical properties of copper nanoparticles prepared by a chemical reduction method," *Adv. Nat. Sci. Nanosci. Nanotechnolo.*, vol. 2, pp. 015009, March 2011.

[10] A.A. Ponce, and K.J. Klabunde, "Chemical and catalytic activity of copper nanoparticles prepared via metal vapour synthesis," *Journal of Mol. Catal. A*, vol. 225, pp. 1-6, Jan. 2005.

[11] Z. Huang, F. Cui, H. Kang, J. Chen, X. Zhang, and C. Xia, "Highly dispersed silica supported copper nanoparticles prepared by precipitation – gel method : a simple but efficient and stable catalyst for glycerol hydrogenolysis," *Chem. Matter.*, vol. 20, pp. 5090-5099, Jul. 2008.

[12] R.A. Soomro, S.T.H. Sherazi, N. Sirajuddin Memon, M.R. Shah, N.H. Kalwar, K.R. Hallam, and A. Shah, "Synthesis of air stable copper nanoparticles and their use in catalysis," *Adv. Mat. Let.*, vol. 5, no. 4, pp. 191-198, Apr. 2014.

[13] Kiranmai Mandava, Keerthi Kadimcharla, Neeraja R. Keesara, Sumayyan N. Fatima, Prathyusha Bommena, and Uma R. Batchu, "Green synthesis of stable copper nanoparticles and synergistic activity with antibiotics," *Indian J. Pharm. Sci.*, vol.79, No.5, pp. 695-700, July 2017.

[14] M. Mano Priya, B. Karunai Selvia, and J.A. John Paul, "Green synthesis of silver nanoparticles from the leaf extracts of *Euphorbia hirta* and *Nerium indicum*," *Digest J. Nanomat. Biostruct.*, vol. 6, pp. 869-877, May 2011.

[15] Chandrakant K. Tagad, D. Sreekantha Reddy, A. Rohini, P. Sungha, K. Atul, and S. Sushma, "Green synthesis of silver nanoparticles and their application for the development of optical fiber based hydrogen peroxide sensor," *Sens Actuators B Chem.*, vol. 183, pp. 144-149, July 2013.

[16] G. Yamini Sudha Lakshmi, B. Fouzia, Ezhilarasan, Arumugam, and Sahadevan, "Green synthesis of silver nanoparticles from *Cleome viscose* : synthesis and antimicrobial activity," International Conference on Bioscience, Biochemistry and Bioinformatics, IPCBEE, @ IACSIT Press, Singapore, vol. 5, pp. 334-337, 2011.

[17] Won-Kyu Han, Jae-Woong Choi, Gil-Ho Hwang, Seok-Jun Hong, Jai-Sung Lee, and Sung Goon Kang, "Fabrication of Cu nanoparticles by direct electrochemical reduction from CuO nanoparticles," *App. Surf. Sci.*, vol. 252, pp. 2832-2838, Feb. 2006.

[18] Ill-Min Chung, Abdul Abdul Rahuman, Sampath Marimuthu, Arivarasan Vishnu Kirthi, Karunanithi Anbarasan, Parthasarathy Padmini, and Govindasamy Raja Kumar, "Green synthesis of copper nanoparticles using *Eclipta prostrate* leaves extract and their antioxidant and Cytotoxic activities," *Experimental and Therapeutic medicine*, vol. 14, pp. 18-24, July 2017.

[19] B. Ankamwar Ed., "Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution," *Journal of Nanoscience & Nanotechnology*, vol.5, no.10, pp.1665-1671, 2005.

[20] H.J. Lee Ed., "Biological synthesis of copper nanoparticles using plant extract," *Nanotechnology*, no.1,pp. 371-374, 2011.

[21] H. Bar Ed, "Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*," *Colloids & Surfaces*, vol.348, no.1-3, pp. 212-216, 2009.

[22] M. Sathishkumar Ed., "Cinnamon Zeylancium bark extract & powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity," *Colloids & Surfaces*, vol.73, no.2, pp. 332-338.

[23] VVT. Padil, and M. Cernik, "Green synthesis of copper oxide nanoparticle using gum karaya as a biotemplate and their antibacterial application," *Int. J. Nanomed.*, vol. 8, no. 1, pp. 889-898, Feb. 2013.

[24] D. Das, B.C. Nath, P. Phukon, and S.K. Dolui, "Synthesis and evaluation of antioxidant and antibacterial behaviour of CuO nanoparticles," *Colloids Surf B. Biointerfaces*, vol. 101, pp. 430-433, Jan. 2013.

[25] M. Heinlaan, A. Ivask, I. Blinova, HC. Dubourguier, and A. Kakru, "Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria *Vibrio fischeri* and Crustaceans *Daphnia magna* and *Thamnocephalus platyurus*," *Chemosphere*, vol. 71, no. 7, pp. 1308-1316, April 2000.

[26] S.Iravani, "Green synthesis of metal nanoparticles using plants," *Green Chemistry*, vol.13, pp. 2638-2650, 2011.

[27] M. Gopinath, R. Subbaiya, M. Masilamani Selvam, and D. Suresh, "Synthesis of copper nanoparticles from *Nerium oleander* leaf extract and its antibacterial activity," *Int. J. Curr. Microbiol. App. Sci.*, vol. 3, No. 9, pp. 814-818, 2014.

[28] Sundararaj, "Practical Manual of Microbiology," IIIrd (ed.), pp. 27-50, 1997.

[29] R. Allaker, "The use of nanoparticles to control oral biofilm formation," *J. Dent. Re.*, vol.89, no. 11, pp. 1175-1186, Aug. 2010.

[30] G. Sanchez-Sanhueza, D. Fuentes-Rodriguez, and H. Bello-Toledo, "Copper nanoparticles as potential antimicrobial agent in disinfecting root canals. A Systematic review," *Int. J. Odontostomat.*, vol.10, no.3, pp. 547-554, Dec. 2016.

[31] Vinod Vellora, Thekkar Padil and Miroslav Cernik, "Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial applications," *International Journal of Nanomedicine*, vol.8, pp. 889-898, 2013.

[32] Asim Umer, Shahid Naveed and Naveed Ramzan, "Selection of a suitable method for the synthesis of copper nanoparticles," *NANO: Brief Reports & Reviews*, vol.7, no.5, pp. 1-18.

[33] A. Nasirian, "Synthesis and Characterization of Cu nanoparticles and studying of their catalytic properties," *International Journal of NanoDimension*, vol.2, no.3, pp.159-164.

[34] S. Honary, H. Barabadi, E. Gharaei-Fathabad, and F. Naghibi, "Green synthesis of copper oxide nanoparticles using *Penicillium aurantiogriseum, Penicillium citrinim & Penicillium waks-manii*," *Dig. J. Nanomat. Biostruct.*, vol. 7, pp. 999-1005, Sep. 2012.

[35] M, Yang, J. He, M. Hu, H. Xiaochun, Y. Chunxiao, and Z. Cheng, "Synthesis of copper oxide nanoparticles and their sensing property to hydrogen cyanide under varied humidity conditions," *Sensor Actuat. B-Chem.*, vol. 213, pp. 59-64, July 2015.

[36] S. Karthikarani and G. Suresh, "Green synthesis, Characterization and its antibacterial activity of copper oxide nanoparticles using *Ocimum sanctum Piper nigrum* extract," *International Journal for Research in Applied Science & Engineering Technology*, vol.5, no.12, pp.411-419.

 DOI : 10.18231/2454-9150.2018.1220