Preparation of herbal formulation and its application on nanoparticles synthesis and antibacterial activity

Srijan Sunar, Rajeshkumar S*, Anitha Roy, Lakshmi T

Department of Pharmacology, Nanobiomedicina Lab, Saveetha Dental College, Saveetha University, SIMATS, Chennai-600077, Tamil Nadu, India

Article History:
Received on: 12.03.2019
Revised on: 19.06.2019
Accepted on: 23.06.2019

Keywords:
Copper nanoparticles, Staphylococcus aureus, toothpaste, oral medicine, nanotechnology

ABSTRACT
Copper nanoparticles makes important progress in the area of nanotechnology and nanomedicine due to their good optical, electrical and anti-fungal/bacterial application. It is prepared using some methods such as vacuum vapour deposition, microwave irradiation methods, chemical reduction and laser ablation. The chemical reduction method is simple, inexpensive and gives a liable control of geometrical nanoparticle characteristics like size and shape. 20 millimolar of 80 ml copper sulphate prepared using double distilled water. The plant extract is added with the metal solution and was made into 100 ml solution. The synthesised nanoparticles solution is preliminarily characterized by using UV-vis-spectroscopy, 3ml of the solution is taken in cuvette and scanned in double beam UV-vis-spectrophotometer from 300 nm to 700 nm wavelength. The agar well diffusion method is used. Different concentration of Cu NPs was tested against Staphylococcus aureus, Streptococcus mutans (gram +), and Pseudomonas sp. The result reveals that Moringa Oleifera with Andrographis paniculata formulation mediated with copper nanoparticles show effective antibacterial activity. CuNPs ex significantly higher activity with an increase in the zone of inhibition diameter. The plant extract is observed to be dark green, and the copper nanoparticles are seen to be in light greenish in colour. They can be used in toothpaste and oral medicines due to their antibacterial activity. Nanoparticles are expected to be used in future for the effective drug systems and immunity against diseases.

INTRODUCTION
Copper nanoparticles makes important progress in the area of nanotechnology and nanomedicine for a long time due to their good optical, electrical and anti-fungal/bacterial application (Tiwari et al., 2016; Karthiga et al., 2018a; Rajeshkumar et al., 2018b). It is prepared using some methods such as vacuum vapour deposition, microwave irradiation methods, chemical reduction and laser ablation (Karthiga et al., 2018b; Rajeshkumar, 2018; Rajeshkumar et al., 2018a). Among all the methods, the chemical method is found to be the best method in preparation of CuNPs. The chemical reduction method is simple, inexpensive and gives a liable control of geometrical nanoparticle characteristic, i.e., size and shape. It also has disadvantages like it has some effect over the environment, because of consumption and elimination of toxic particles or hazardous chemical reducing agents (Rajeshkumar et al., 2018c). Copper nanoparticles can serve as potential candidates for...
the application such as nanomedicine, water treatment and food processing (Perreault et al., 2012).

Valodkar reported biosynthesis of copper nanoparticles, using the plant Euphorbia nivulia and exhibit biological effects on tumour cell (Grigore et al., 2016; Sivaraj et al., 2014a). In recent years, the nanoparticles are used in various fields like waste water treatment, environmental remediation, consumer products, medical pharmaceutical, energy and electric industries. The chemical properties of synthesised Copper oxide nanoparticles are characterised by analytic techniques.

**MATERIALS AND METHODS**

**Synthesis of nanoparticles**

20 millimolar of 80 ml Copper sulphate solution prepared using double distilled water. The Herbal formulation is added with the metal solution and was made into a 100ml solution. The colour change was observed visually, and photographs were recorded. The solution is kept in magnetic stirrer/ orbital shaker for nanoparticle synthesis.

**Figure 1: Visual observation (a) Plant Extract (b) CuNPs**

**UV-vis spectrophotometer**

![Figure 2: UV-vis spectrum of CuNPs](image)

**Characterisation of nanoparticles**

The synthesised nanoparticles solution is preliminarily characterised by using UV-vis-spectroscopy, 3ml of the solution is taken in cuvette and scanned in double beam UV-vis- spectrophotometer from 300 nm to 700 nm wavelength. The results were recorded for the graphical analysis. The prepared nanoparticles powder is morphologically analysed using a transmission electron microscope.

**Antibacterial activity of nanoparticles against oral pathogens**

The agar diffusion method is used to demonstrate the antibacterial action of copper nanoparticles. Different concentration of Cu NPs is tested against Staphylococcus aureus, Streptococcus mutans (gram +), and Pseudomonas sp. The new bacterial suspension is dispersed over the surface of the Muller Hinton agar plate. Many concentration of nanoparticles (20, 40 & 60 μL) is incorporated into the wells and plate is incubated at 37°C for 24 hours. The antibiotics are utilised in positive control. Zone of inhibition is recorded in each plat.

**RESULTS AND DISCUSSION**

**Visual observation:**

The plant extract is observed to be dark green, and the copper nanoparticles are seen to be in light greenish in colour (Figure 1), the colour change indicates the nanoparticles synthesis (Gunalan et al., 2012; Guidelli et al., 2011)

**UV visual spectroscopy**

Ultra violet spectra of Copper oxide particles synthesised plant extract at room temperature. It is generally recognised that UV-vis spectra can be used in the examination of the size and shape-controlled
nanoparticles in the aqueous solution with 200-800nm wavelength range (Phiwdang et al., 2013; Ananth et al., 2015). The particle shape is spherical and size in a range of 2-100nm. The graph reached its peak at the wavelength of 300nm. The optical absorption spectra of noble metal nanoparticles is found to exhibit excellent optical properties due to the phenomenon of surface plasmon resonance, which proceeds to longer wavelength and increasing particle size simultaneously (Figure 2).

**Antibacterial activity**

Antibacterial activity of copper nanoparticles is against gram-positive and gram-negative bacteria (Figure 3). A copper nanoparticle has an efficient and bactericidal effect against oral pathogens. The growth inhibition of cells is because of the distraction of cell membrane by copper oxide nanoparticles, which leads to break down of cell enzymes (Dagher et al., 2014; Sivaraj et al., 2014b). The result reveals that the *Moringa Oleifera* mediated with copper nanoparticles show powerful antibacterial activity.

CuNPs exhibition significantly higher activity with an increase in the zone of inhibition diameter.

**CONCLUSIONS**

Nanoparticles due to its antibacterial property and efficient effect against the oral pathogens the is used in toothpaste and oral medicines. Hence the nanoparticles are expected to be used in future for the effective drug systems and immunity against diseases.

**REFERENCES**

Ananth, A., Dharaneedharan, S., Heo, M. S., Mok, Y. S. 2015. Copper oxide nanomaterials: Synthesis, characterization and structure-specific antibacterial performance. Chemical Engineering Journal, 262:179–188.

Dagher, S., Haik, Y., Ayesh, A. I., Tit, N. 2014. Synthesis and optical properties of colloidal CuO nanoparticles. Journal of Luminescence, 151:149–154.

Grigore, M. E., Biscu, E. R., Holban, A. M., Gestal, M. C., Grumezescu, A. M. 2016.

Guidelli, E. J., Ramos, A. P., Zaniquelli, M. E. D., Baffa, O. 2011. Green synthesis of colloidal silver nanoparticles using natural rubber latex extracted from Hevea brasiliensis. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 82(1):140–145.

Gunalan, S., Sivaraj, R., Venckatesh, R. 2012. Aloe barbadensis Miller mediated green synthesis of mono-disperse copper oxide nanoparticles: Optical properties. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 97:1140–1144.

Karthiga, P., PonnaniKajamiedeen, M., Rajendran, R. S., Annadurai, G., &amp; S Rajeshkumar 2018a. Characterization and toxicology evaluation of zirconium oxide nanoparticles on the embryonic development of zebrafish, Danio rerio. Drug and Chemical Toxicology, 42(1):104–111.

Karthiga, P., Rajeshkumar, S., Annadurai, G. 2018b. Mechanism of Larvicidal Activity of Antimicrobial Silver Nanoparticles Synthesized Using Garcinia mangostana Bark Extract. Journal of Cluster Science, 29(6):1233–1241.

Perreault, F., Melegari, S. P., Costa, C. H. D., Rossetto, A. L. D. O. F., Popovic, R., Matias, W. G. 2012. Genotoxic effects of copper oxide nanoparticles in Neuro 2A cell cultures. Science of The Total Environment, 441:117–124.

Phiwdang, K., Suphankij, S., Mekprasart, W., Pecharapa, W. 2013. Synthesis of CuO Nanoparticles by Precipitation Method Using Different Precursors. Energy Procedia, 34:740–745.

Rajeshkumar, S. 2018. Synthesis of Zinc oxide nanoparticles using algal formulation (Padina tetrastromatica and Turbinaria conoides) and their antibacterial activity against fish pathogens. Research Journal of Biotechnology, 13(9):15–19.

Rajeshkumar, S., Agarwal, H., et al. 2018a. One-Pot Synthesis of Zinc Oxide Nanoparticles Using Orange Peel Extract and Its Potential Anti-Bacterial Activity. International Journal of Pharmaceutical Research, 10(3):574–578.

Rajeshkumar, S., Agarwal, H., Kumar, S. V., Lakshmi, T. 2018b. Brassica oleracea Mediated Synthesis of Zinc Oxide Nanoparticles and its Antibacterial Activity against Pathogenic Bacteria. Asian Journal of Chemistry, 30(12):2711–2715.

Rajeshkumar, S., Kumar, S. V., Ramaiah, A., Agarwal, H., Lakshmi, T., Roopan, S. M. 2018c. Biosynthesis of zinc oxide nanoparticles using Mangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzyme and Microbial Technology, 117:91–95.

Sivaraj, R., Rahman, P. K. S. M., Rajiv, P., Narendhran, S., Venckatesh, R. 2014a. Biosynthesis and characterization of Acalypha indica mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 129:255–258.
Sivaraj, R., Rahman, P. K. S. M., Rajiv, P., Narendhran, S., Venckatesh, R. 2014b. Biosynthesis and characterization of Acalypha indica mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 129:255–258.

Tiwari, M., Jain, P., Hariharapura, R. C., Narayanan, K., Bhat, K., Udupa, U., Rao, N., ., J. V. 2016. Biosynthesis of copper nanoparticles using copper-resistant Bacillus cereus, a soil isolate. Process Biochemistry, 51(10):1348–1356.