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Research Article

Formulation and Characterization of Copper Nanoparticles Using *Nerium odorum* Soland Leaf Extract and Its Antimicrobial Activity

Anand Kumar Srivastava* **and Dwivedi KN**

Department of Dravyaguna, Faculty of Ayurveda, IMS, Varanasi, India

***Corresponding author:** AAnand Kumar Srivastava, Department of Dravyaguna, Faculty of Ayurveda, IMS, Varanasi, India, Tel: +9415624692; E-mail: dr_aksrivastava.phe@itbhu.ac.in

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Abstract

Nanoparticles, compared to bulk materials, exhibit improved characteristics due to their size, distribution and morphology and are widely used in numerous scientific fields. Among metallic nanoparticles, Copper Nanoparticles (CuNPs) are very important mainly due to their physiochemical and antimicrobial properties which help in therapies, molecular diagnostics and in devices used for medical procedures. An eco-friendly method has been reported for the synthesis of Copper Nanoparticles (CuNPs) using extract of *Nerium odorum* Soland plant leaf that acts as a reducing agent, and it is non-toxic and cheap. UV-Visible spectrophotometer analysis has confirmed the formation of CuNPs by showing a typical resonance (SPR) at about 580-610 nm which is specific for CuNPs. The synthesized nanoparticles were characterized using Particle Size Analysis (139.2 nm), Fourier Transform Infrared (FTIR) Spectroscopy and Scanning Electron Microscope. These results confirm the formation of CuNPs. Since fungal infection is a common problem so the antifungal activity of CuNPs has been checked against *Candida albicans*. Zone of inhibition has been observed. So these nanoparticles formulated using plant extracts have the potential to serve as possible eco-friendly alternatives to chemical and physical methods for fungal infections.

Keywords: Copper nanoparticles; *Nerium odorum* Soland; Antibacterial; Antifungal activity

Introduction

Fungi are present everywhere. There are billions of different fungal species present on Earth, but only around 300 of those are known to make people sick [1]. Fungal diseases are usually caused by fungi that are common in the environment. Fungi are present in soil and on plants and trees as well as on many indoor surfaces and human skin. Most of the fungi are not dangerous, but some types of fungi can be harmful to health [2]. These fungi grow very fast when they find a suitable environment to grow. It is difficult to control fungal growth because fungi have developed resistance to many conventional fungicides such as benzimidazoles and dicarboximides [3]. To overcome this resistance, it is essential to explore novel antifungal agents, which may replace current control strategies.

In recent years, Nanoparticle (NP) materials have received increasing attention due to their unique physical and chemical properties. They differ significantly from their conventional counterparts [4]. Recent studies have demonstrated the antimicrobial activities of various NP materials, including silver, copper [5] and zinc oxide [6].

Copper Nanoparticles (CuNPs) have attracted many people because of their low-cost and novel optical, mechanical, catalytic, electrical and thermal conduction properties, which are different from their bulk metals. Since there are various applications, there has been a particular focus on the synthesis of CuNPs for the past few decades. Green synthesis of Copper nanoparticles is a new approach towards its synthesis as it is Eco-friendly. Many articles reported that plant like *Musa sapientum* (Dinesh et al.) [7], *Clerodendrum inerme* (Arshad et al.) [8], *Coriandrum sativum*, *Cleome viscosa*, *Saururus chinenis*, and many others are used for the synthesis of Nanoparticles. *Nerium odorum* Soland is an evergreen shrub or small tree in the dogbane family *Apocynaceae*, toxic in all its parts. It is the only species currently classified in the genus *Nerium* [9].

Nerium odorum Soland is an evergreen shrub or small tree in the dogbane family *Apocynaceae*, toxic in all its parts. It is the only species currently classified in the genus *Nerium* [9]. Herein the leaf extract of *Nerium odorum* Soland is used for the synthesis of Copper Nanoparticles. Previously *Nerium odorum* Soland was found to be cardio tonic; antibacterial, anticancer and antiplatelet aggregation activities and also its depression in the central nervous system have been reported from the species of *Nerium odorum* Soland. Flavonoids are a class of compounds isolated in large quantities from the genus of *Nerium*. Flavonoids account for approximately two-thirds of the phenolics in our diet as well as a high percentage of the secondary metabolites in *Nerium odorum* Soland [10]. There is also a report on the antioxidant activity of essential oil of *Nerium odorum* Soland flower. *Nerium odorum* Soland consists of many phenolic compounds with other compounds that have a tendency for oxidation. Hence this plant acts as a reducing agent. This plant extract easily reduces copper compounds, and as a resultant we get CuNPs.

Materials and Methods

Collection of plant leaves

Nerium odorum Soland leaves were collected from the lawn of Rajputana Hostel IIT (BHU), Varanasi. The collected leaves were tightly packed with polythene bag and then transfer to the laboratory on the same day. Then leaves were washed with distilled water twice and kept under room temperature for two weeks in dark condition so that their phytochemical constituents did not degrade. After two weeks when leaves were completely dried. They were made into powder using blender.

Preparation of plant leaf extract

The powder of *Nerium odorum* Soland leaf was weighed 5 g and dissolved in 100 ml of distilled water and boiled for 20 min at 50°C with constant stirring. The extract is filtered by Whatmann No 1 filter Paper. Then the filtrate is stored in a tight seal pack under 4°C for further use.

Preparation of 1 mM solution of copper (II) sulphate solution

24.96 mg of Copper (II) sulphate petahydrate salt was weighed using Digital electronic balance, and it was dissolved in water and volume was made up to 100 ml.

Synthesis of copper nanoparticles

For a reaction mixture, 80 ml of 1 mM $CuSO_4.5H_2O$ solution was taken and was stirred using magnetic stirrer at 2000 rpm. To the solution of Copper (II) Sulphate solution 20 ml of Plant, leaf Extract was added slowly using syringe. The reduction of Cu^{2+} was indicated by colour change from light colour to dark colour. Here extract of *Nerium odorum* Soland acts as a reducing agent.

Characterization of copper nanoparticles

UV-Visible spectrophotometer analysis: The synthesized copper Nanoparticles were characterized through UV-Vis spectrophotometer (Shimandzu (1700), Double beam, Japan). The reduction of copper Nanoparticles was monitored by UV spectrophotometer range of absorbance from 350-850 nm.

Particle size analysis: Copper nanoparticles obtained extract of *Nerium odorum* Soland was diluted with filtered ultrapure water (0.22 μm) before the particle size analysis. Diluted Nano dispersion was subjected to particle size using Photon Correlation Spectroscopy (PCS) DelsaNano C (Beckman Coulter Counter, USA) Particle Size Analyser. The instrument measures the fluctuation rate of laser light due to the particle diffusion in fluid and interprets it as size. All particle size measurements were performed in triplicate using a scattering angle of 90° and at 25°C.

Fourier Transform Infrared (FTIR) spectroscopy: Copper Nanoparticles formed using extract of *Nerium odorum* Soland plant leaves was poured into a petridish and kept in a hot air oven until it got dried off, after that the dried sample was scrubbed, powder form of the sample was stored in a sterile Eppendorf. Then the given sample in powder form was used for the FT-IR analysis (Shimadzu, Model 8400S, Tokyo, Japan). FT-IR spectra of Copper Nanoparticles and oxidized extract of *Nerium odorum* Soland was obtained by the conventional KBr Disc/Pellet method. The sample was prepared by grinding with anhydrous KBr powder and compressing them to form pellets. The FT-IR spectra were measured over the range of (4000-400 cm^{-1}) with resolution range of 4 cm^{-1} for 50 scans.

Scanning electron microscopy: Surface morphology of CuNPs was visualized using scanning electron microscope under normal atmospheric conditions. The scanning electron microscope (ZEISS Supra 40, Germany) was used at 5 kV. The sample was analysed in SEM at high vacuum in varying magnifications. For scanning electron microscopy the sample was dropped onto glass slides, spread evenly and then followed by vacuum drying.

Antifungal activity: To check the antifungal activity of Copper Nanoparticles formed by extract of *Nerium odorum* Soland, the freshly prepared sample was made and samples were given to Microbiology Lab of Institute of Medical Science (Banaras Hindu University) Varanasi.

A fungus *Candida albicans* was taken from the stock culture and dissolved in 25 ml of Nutrient broth kept for incubation at 12 hrs. The synthesized copper nanoparticles using *Nerium odorum* Soland leaf extract was tested for antifungal activity by agar well-diffusion method against Candida Albicans. Then incubated cultures of fungus were swabbed uniformly on the plates using sterile cotton swabs on the Muller Hinton Agar. Three wells were made of 6 mm in diameter on Muller Hinton Agar plates with the help of gel puncture. Using a micropipette, 30 μl of the synthesized copper nanoparticle synthesized silver nanoparticle (another student) and extract of *Nerium odorum* Soland was put in the well, Plates were incubated at 37°C for 24 hrs to observe the formation of zone of inhibition.

Results and Discussion

Synthesis of copper nanoparticles (Visual inspection)

After 24 hrs of reaction, the reaction mixture colour change from Light to dark colour that can be given below Figure 1. The reduction of Cu+ ions leads to the formation of CuNPs and this nanoparticle exhibit the dark colour due to the excitation of Surface Plasmon vibration in metal nanoparticles.

The synthesized copper Nanoparticles were characterized through UV-Vis spectrophotometer (Shimandzu (1700), Double beam, Japan). The reduction of copper Nanoparticles was monitored by UV-spectrophotometer range of absorbance from 350-850 nm. From the graph (Figure 2) we can easily determine the peak that indicates the surface Plasmon resonance (SPR), in this case, we got peak at 580 nm to 610 nm which determines the formation of copper nanoparticles.

FTIR spectroscopy, a type of vibrational spectroscopy, is used to identify the stretching and bending frequencies of molecular functional groups attached to Cu NP surface [11]. Figure 3 reveals the FTIR spectra of copper NPs. These spectra reveal some absorption peaks at different IR frequencies. The absorption at distinct frequencies is related to several various modes of interatomic bond vibration (stretching or bending) and degrees of Hydrogen bonding [12]. In our FTIR report, we got various characteristics peaks which are assigned to different stretching and bending frequencies of molecular functional groups attached to CuNP surface.

3396.76, 3369.75-These are broad and strong band which ascribed to O-H (hydroxyl) stretching vibrations on the surface of copper NPs. They are the most intense and easily recognizable bands in the spectra, and their precise position depends on the strength of the hydrogen bonds [13-16]; 2928.04-It originates from C-H (hydrocarbon) stretching vibrations in the molecules [16]; 2285.72-It originates from Cu-H stretching; 1610.61-It is the characteristic of bending vibration of O-H (hydroxyl) bonds in OH groups [17]; 1433.16-It gives information about the bending vibration of C-H bonds resulting from CH_2 in chemical structure [15]; 1265.35, 1051.24-It indicates C-O stretching; 698.25, 607.60, 536.23-They all indicates Cupper oxygen bond [15]. All these peaks indicate the formation of Copper Nanoparticles.

Particle size analysis

Particle size and polydispersity index of Copper Nanoparticles were measured by Photon Correlation Spectroscopy (PCS) DelsaNano C (Beckman Coulter Counter, USA) Particle Size Analyser. The average particle size of the copper nanoparticle was found to be 139.2 nm (Figure 4). The particle size range of 50 to 1000 nm is generally considered as nanoparticles. This indicates the particle size was in the nano range.

Figure 1: Copper Nanoparticles.

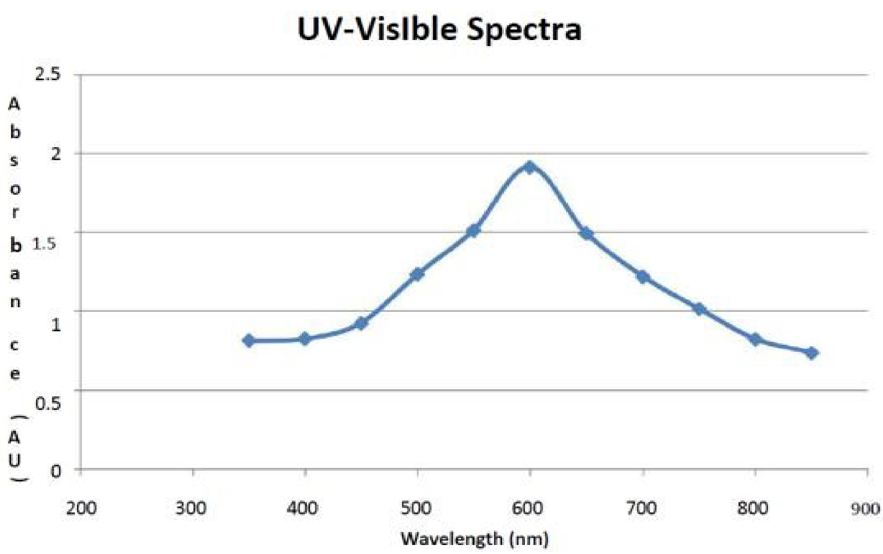


Figure 2: Absorbance of Copper nanoparticles at different wavelength.

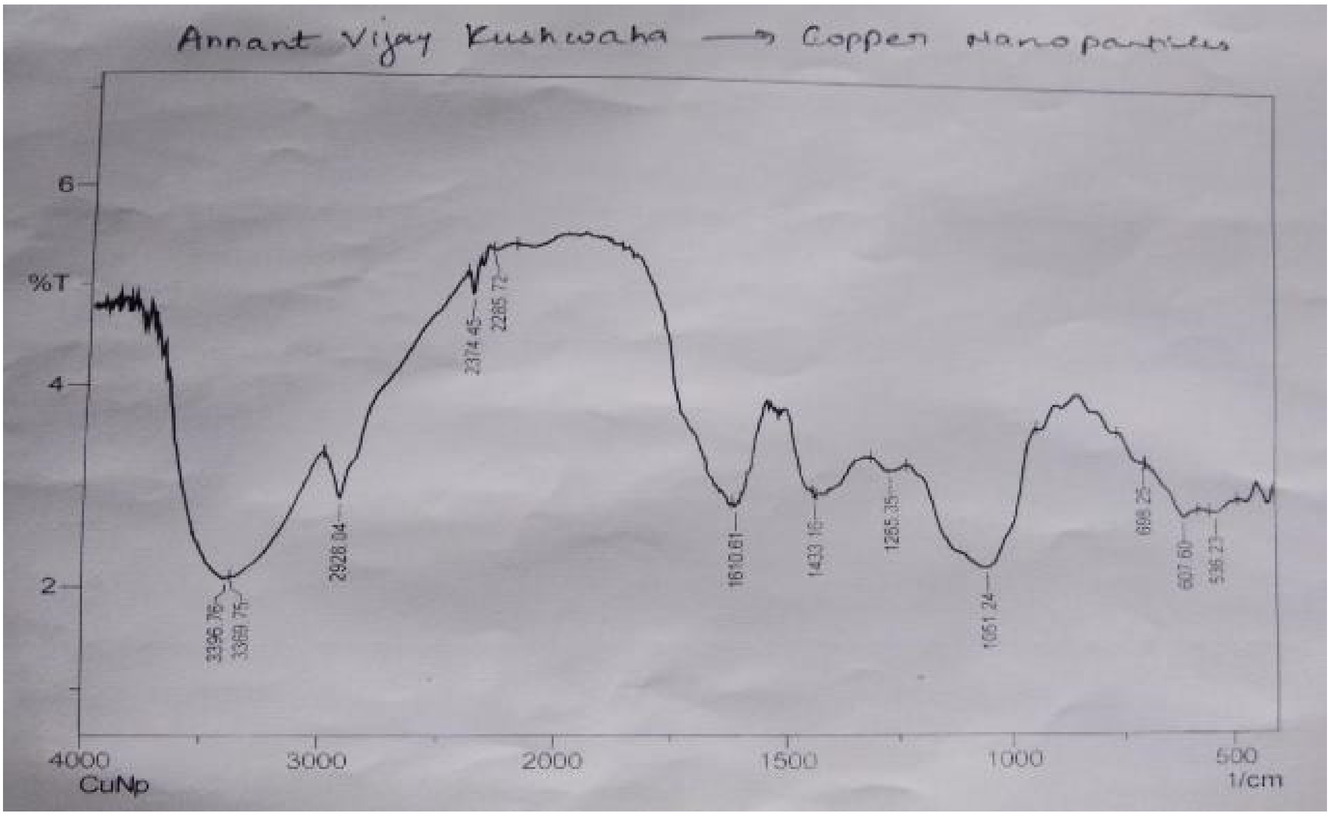


Figure 3: FTIR of Copper Nanoparticles.

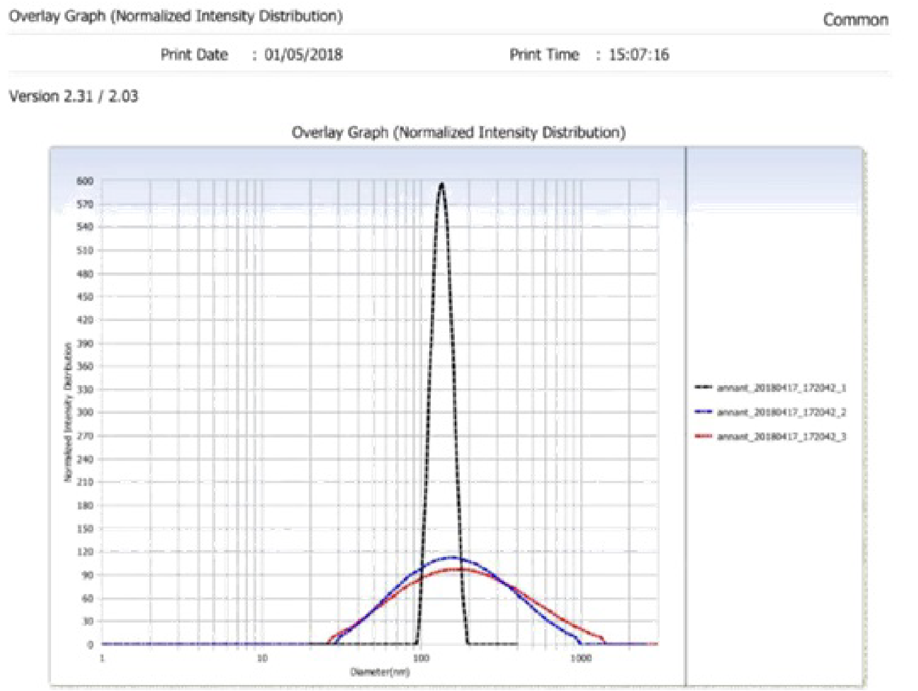
Polydispersity index of Copper Nanoparticles was found to be 0.341 (Figure 5).

Scanning electron microscopy

Surface morphology of CuNPs was visualized using scanning electron microscope under normal atmospheric conditions. The scanning electron microscope (Zeiss Supra 40, Germany) was used at 5 kV.

The sample was analysed in SEM at high vacuum in varying magnifications. For scanning electron microscopy the sample was dropped onto glass slides, spread evenly and then followed by vacuum drying.

Figure 4: Particle size of Copper nanoparticles.



No	Data	Repet. No	pH	Ave. Diameter(nm)	Polydispersity Index	D (10%) (nm)	D (50%) (nm)	D (90%) (nm)
1	annant_20180417_172042_1	1	NA	142.4	-0.178	109.4	131.7	158.9
2	annant_20180417_172042_2	2	NA	132.8	0.291	58.0	153.4	423.0
3	annant_20180417_172042_3	3	NA	142.4	0.341	56.0	170.0	549.3

Figure 5: Normalised Intensity distribution of Copper nanoparticles.

The following SEM Images of Copper Nanoparticles were obtained (Figures 6 and 7).

Antibacterial activity

The antibacterial activity of *Nerium odorum* Soland was evaluated against *Staphylococcus aureus* by using Agar disk diffusion method (Table 1). The zone of inhibition was found to be 22 mm (Figure 8).

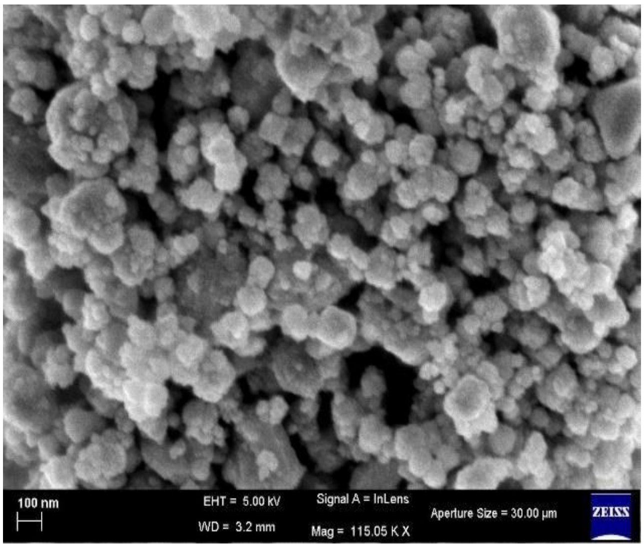


Figure 6: SEM of Copper Nanoparticles at 100 nm scale.

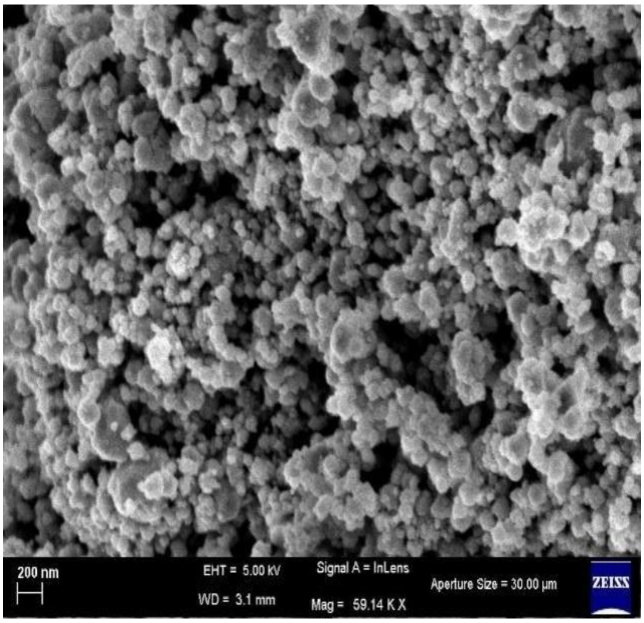


Figure 7: SEM of Copper Nanoparticles at 200 nm scale.

Bacteria	Type
S. aureus	Gram +ve
B. subtillis	Gram +ve
B. pumillus	Gram +ve

Table 1: Bacterial culture.

Figure 8: Antibacterial Activity of Copper Nanoparticles.

Figure 9: Antifungal Activity of Copper Nanoparticles.

Antifungal activity

The antifungal activity of Copper Nanoparticles prepared using *Nerium odorum* Soland plant leaf extract was evaluated against *Candida albicans* using Agar disk diffusion method. The zone of inhibition was found to be 21 mm (Figure 9).

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