



Studies on Anti- bacterial activity of newly synthesized copper Nano particles from *Artimisia pallens L.*

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Abstract:

The the present investigation, CuNPs were synthesized by using an aqueous *Artimisia pallens L.* hole plant extract (APHPE) to form an *Artimisia pallens* hole plant extract copper nanoparticle (APHPECuNPs or CuNPs) and corroborated from the X-ray diffraction spectrum. As-synthesized CuNPs were spherical with an average particle size of ~22 nm. Fourier transform infrared spectrum suggested capping of the phyto constituents[1-3], probably polyphenols which might appear from the APHPE. The results obtained from the antimycobacterial activity assays suggested that these green APHPECuNPs were more potent against *E. coli*.

Keywords: *A. pallenes*; copper nanoparticles; bacterial activity; haemolytic assay; MIC, antioxidant activity

1. Introduction

Tuberculosis (TB) is one of the commonly known diseases occurred worldwide due to which about 9.4 million case were newly registered and about 1.7 million were reported as dead in 2009. TB is a chronic infectious disease caused by several species of mycobacteria and where deadly bacterial pathogen of *M. tuberculosis* is the causative agent. Due to multidrug resistant-strains of mycobacteria and to a high prevalence of tuberculosis in patients who have Acquired Human Immunodeficiency Syndrome (AIDS), treatment of HIV-related TB is complex as there is overlapping of drug toxicities and drug-drug interactions between antiretroviral treatment and anti-TB treatment. This causes the risk for development of immune reconstitution inflammatory disease. There is need to develop a targeted drug delivery system for

tuberculosis for improving patient's compliance. Functionalized copper nanoparticles (Cu NPs)-based drug delivery system which has high affinity towards infected tubercle cells than normal cells. These NPs are being extensively applied in surgical instruments, wound dressings, bond prostheses and heart valves, electronics, and biosensing . The literature survey revealed that the Cu NPs synthesized by using environmentally clean i.e. green route produce better results as compared to that of synthesized from a chemical base.

In the present investigation, using plant antitubercle agent source, synthesis of Cu NPs is reported using green approach. These Cu NPs were targeted on three different *Mycobacterium* species to develop novel antituberculosis agents.

2. Materials and method

2.1. Materials

All chemical were of analytical grade. Silver nitrate (99.9%), phosphate buffers, Rifampicin (standard antibiotics) etc., were purchased from Himedia, Latur, and Maharashtra, India.

2.2 Collection of pathogen

The *Mycobacterium tuberculosis* (MTCC 300), *Mycobacterium pheli* (MTCC1723), *Mycobacterium avim* (MTCC 1724) were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh (PB), India. These were subcultured and maintained into Lowenstein-Jensen media.

2.3 The Biosynthesis of Cu NPS

A. Pallens is an alkaloid from the Leguminosae family. The leaves of *A. Pallens*, used in this study, were collected from the Swami Ramanand Teerth Marathwada University Nanded campus, Maharashtra India i.e. ours. These were primarily cleaned with Millipore Deionised (DI) water, washed and dried by pressing with blotting paper and then were shade-dried and chopped into small pieces. Leaves of 10 g were poured into 100 ml of distilled water and irradiated for 5 min with microwaves using ultrasonics cleaner, obtained extract was filtered through Whitman filter paper, and stored at 4°C for further experiments. A 100 ml 0.1 M CuSO₄ was kept in a conical flask wherein 10 ml aqueous *A. Pallens* extract was added while vigorous magnetic stirring. The reduction of Cu⁺ ions into Cu NPs at room temperature was completed in about 30 min, as color of solution was changed from greenish-yellow to dark brown indicating the formation of Cu NPs (Fig. 1). For the purification of Cu NPs the reduced solution was centrifuged at 5000 rpm for 15 min for two to three times. The supernatant liquid was discarded, and the residue was dispersed in distilled water. The residue was

collected, washed with distilled water, dried and stored in vacuum tight chamber for further use. Before the final use, Cu NPs and distilled water was mixed in appropriate proportions for desired Cu NPs concentration.

2.4 Structural and morphological studies

The X-ray diffraction (XRD) measurement was carried out using a structural elucidation X-ray diffraction spectrum obtained from X-ray Diffractometer (Rigaku/MAX 2500 V, Cu K α , $\lambda = 1.5418 \text{ \AA}$). Morphological evolution of Cu NPs was confirmed from the field-emission scanning electron microscopy (FE-SEM, Hitachi S-4200) image. The functional groups present in the phyto-constituents in the leaves extract of *A. Pallens* and their involvement in the synthesis of Cu NPs were confirmed from the FT-IR plot on Nicolet IS-10 (Thermo Fischer Scientific Instruments) for which the Cu NPs were mixed with KBr for forming a pellet.

2.5. Antimycobacterial activity of the test drug

As per experimental standardization, initially 176 mg/100 ml (1M) concentration of NPs was used for antimicrobial analysis which was further taken up to only 20 $\mu\text{l/ml}$ add into the well, however, the clear zone of inhibition was observed under the experimental condition. The sensitivity test of three different strains of *M.tuberculosis*, *M.Pheli*, and *M.avim* was studied by agar diffusion method. In short, a sterile cork borer of 7mm diameter was used to bore holes into the inoculums seeded solidified Lowenstein Jensen medium. A 50 μl volume of Cu NPs was loaded into the well using a sterile pipette. From the CuSO₄ solution (176 mg/100 ml), 150 μl was added in agar well plates. The plates were frizzed for 10 min, useful for an easy diffusion and then incubated at 37° C for 48 h. Growth of *M.tuberculosis*, *M.Pheli*, and *M.avim* was observed after the diameter of the inhibition zone measured the well size. Rifampicin (10 $\mu\text{g/ml}$) was used as reference standard.

2.6 In-vitro stability studies

Cu NPs of 1 ml (176 mg) was incubated at 37°C for 48 h with 0.5 ml of 2-4M saline and PBS (pH 4.5-4.7). Dispersion was preserved for 2, 4, 6, 12, 24, 48 and 72 h and then analyzed spectrophotometrically.



Fig 1. Phyto-reduction Cu^+ to Cu NPs presents change of colour from transparent to brown after 24 h incubation [a) *A. Pallens* leaf extract, B) CuSO_4 c) CuSO_4 with leaf extract].

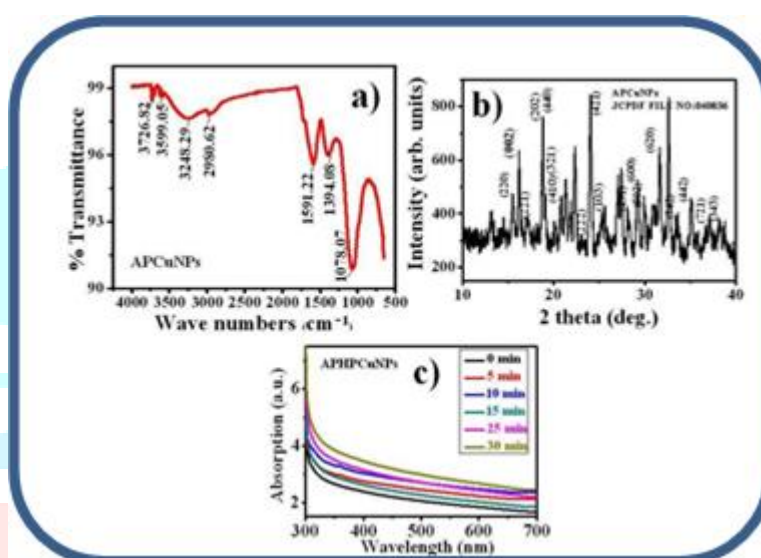


Fig 2. A) Image of FTIR, b) Image of XRD, c) Image of UV

3.2. The antibacterial activity of test drugs

The antimycobacterial activity of three *E coli* was performed against biosynthesized CuNPs. The three *E coli* strains resistant to antibiotics were special and could be a new direction for detection. This effect was dose-dependent and effective against selected *Mycobacterium* strains, similar to others. To assess the antimicrobial activities of the synthesised NPs, standard TB drug such as streptomycin are shown in [Fig. 4 a-c]. The antimycobacterial activity zones, inhibition around the disk of approximately 6 mm each with CuNPs aqueous extract against the *E coli*.

3. Results and discussion

3.1. Structure, bonding morphology studies

In present investigation, Cu NPs were successfully synthesized from plant green leaves. A change of solution color from white to brown, after biotransformation during the biosynthetic process, Furthermore, during overnight incubation in dark room condition, the transparent reaction mixture was turned into a dark brown solution, providing qualitative evidence for the formation of Cu NPs (Fig. 1) [7]. The XRD pattern of the biosynthesized Cu NPs produced by the leaf *A.pallens* extract is presented in (Fig. 3b). The 2θ values at 15.53° , 16.31° , 16.71° , 18.84° , 19.06° , 19.17° , 21.42° , 22.99° , 25.29° , 24.05° , 28.15° , 27.58° , 29.14° , 31.74° , 33.71° , 35.16° , 36.08° , 37.13° were assigned to 220 , 002 , 221 , 202 , 440 , 410 , 321 , 222 , 103 , 421 , 431 , 600 , 502 , 620 , 342 , 442 , 721 , and 343 , reflection planes of Ag (JCPDF file no.400836) (Fig. 3b). FTIR is a valuable tool for knowing the participation of functional groups of metal particles and biomolecules interactions. In the present study, FTIR measurement was performed to identify the biomolecules responsible for capping and stabilizing the Cu NPs. The FTIR spectrum (Fig. 3a) of Cu NPs showed a very strong peak at 1078.07 cm^{-1} corresponding to -OH stretching in alcohols and phenolic compounds. Peaks observed around 1394.08 cm^{-1} , 1391.22 cm^{-1} were assigned to the C-H stretching vibration of methyl, methylene, and methoxy groups. The medium intense peaks at 2980.62 cm^{-1} and 3248.29 cm^{-1} were due to the stretching vibrations of C-O and C-C , respectively. The vibrational bands corresponding to bonds such as -C-C and C-O might be from the compounds such as flavonoids and terpenoids in *A.Pallens* leaf hence, it was presumed that these biomolecules were responsible for capping and stabilization. The absorption band at 3599.05 cm^{-1} was characteristics of amide II (N-H) from proteins, suggesting interaction of proteins with the biosynthesized CuNPs. This result also proved that Cu NPs inhibited the growth of three species *Mycobacterium* species. FESEM (Fig. 3 a-e) images, recorded for two magnifications, clearly present Cu NPs. It was difficult to trace out an exact dimension of this Cu NPs. Agglomerated type nanocrystalline were seen by a normal view whereas irregular void channels were found under high resolution inspection.

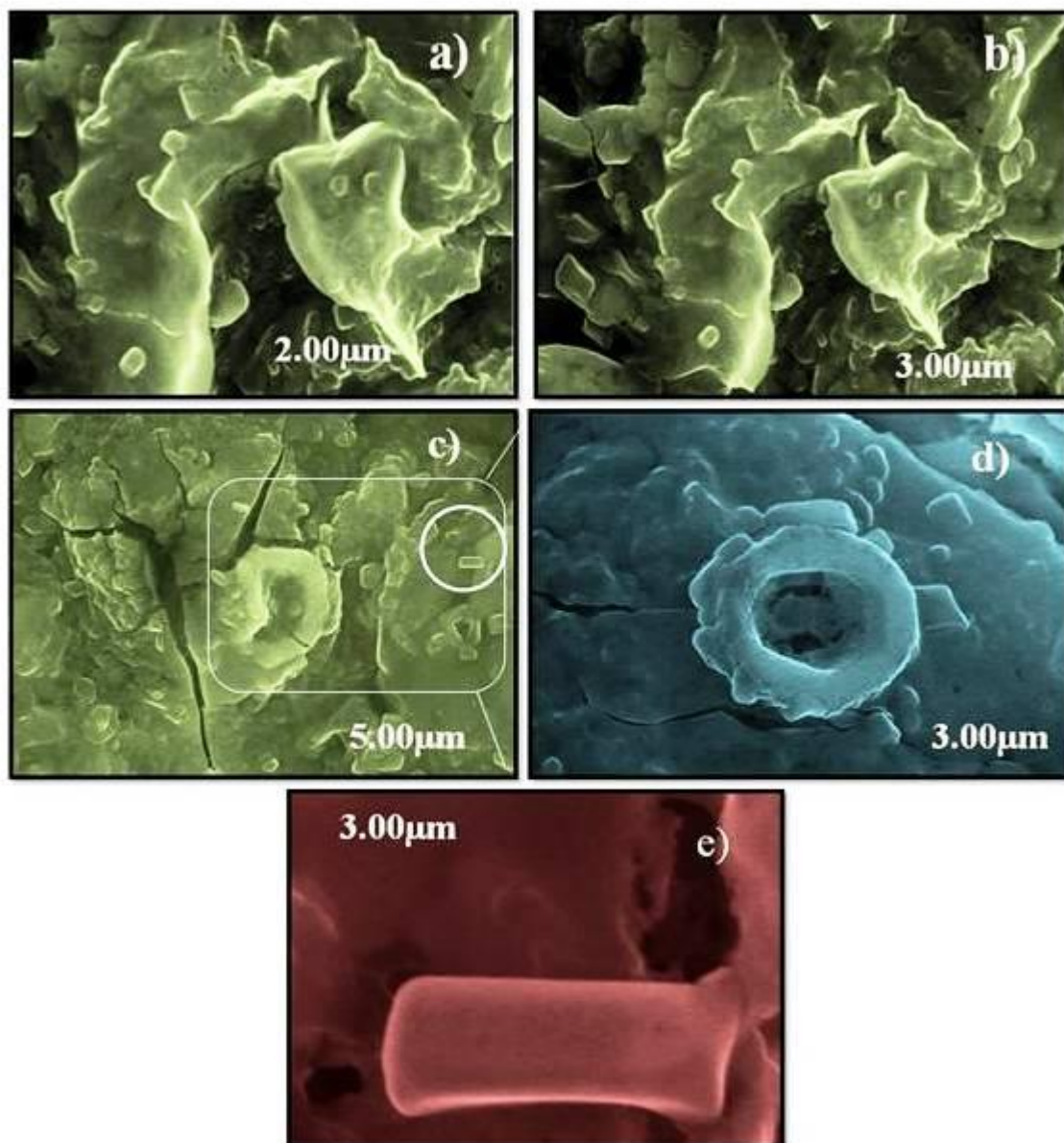


Fig 3. a-e) SEM image of CuNPs

4. Conclusions

In present investigation green route synthesis of bio-functionalized CuSO_4 using *A.Pallens* plant extracts in the presence of sunlight to produce CuNPs. These synthesized Cu NPs are good antimycobacterial agent. Till date, there have been no reports on the as antimycobacterial agent and cytotoxic study of CuNPs from *A.Pallens*. as antibacterial property species *E coli* against CuNPs cause the 99.9% cell death. A direct connection between green approach

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