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A Green perspective on the ability of nanomedicine to inhibit tuberculosis and lung cancer

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Abstract

Tuberculosis is a chronic pulmonary infection that is initiated by Mycobacterium tuberculosis. People suffering from tuberculosis are at an increased risk of getting lung cancer due to inflammation and scarring of lungs, making it a suitable ground for tumour growth. Therefore, there is a need to look out for new drugs that can combat both these chronic diseases with reduced side effects. Plants exhibit chemical diversity and reduced toxicity thereby making them favourable leads to formulate new drugs. In this study nanoparticles of copper (CuNPs) were prepared from the aqueous extract of Coleus aromaticus, characterized, tested for its antimicrobial activity against upper respiratory tract infection causing organisms and its potential to inhibit tuberculosis and lung cancer. The absorbance peak of the nanoparticles was found to be 320 nm, Scanning Electron Microscopy results disclosed that the average particle size varied from 30 – 50 nm and Zeta potential was -5.7 mV. The antimicrobial results showed good zones of inhibition ranging from 10-30 mm. The anti-tuberculosis activity against the Mycobacterium tuberculosis strain H37Rv was done by broth microdilution method and its minimum inhibitory concentration (MIC) was 500 μg/ml. The anti-cancer results showed great inhibition against lung cancer with minimum cytotoxicity to the cells. The results showed that the green nano copper was a potential drug candidate against tuberculosis and lung cancer which are associated chronic debilitating conditions.

1. Introduction

Tuberculosis (TB) which is a persistent lung infection is a major global health concern (Cabrera-Sanchez et al.). The treatment plan involves long term usage of a combination of drugs resulting in side effects such as low efficiency and drug associated liver toxicity (Dyba et al.). Lung cancer claimed the lives of almost 1.8 million individuals in 2020 (Mejri). There is an association between tuberculosis and lung cancer. TB leads to prolonged inflammation of the lungs and therefore those who have the disease are more likely to develop lung cancer (Nabi et al.), (Oh et al.). Nanomedicine is widely used to target cancers.

Nanoparticles are known for their large surface to volume ratios owing to their small size and can be exploited widely. Nanoparticles synthesized from plant sources can be extremely beneficial as they are not only ecofriendly, but also contain innumerable benefits because of the bioactive compounds present in them and would be effective in very small doses.

2. Materials and methods

2.1. Collection of the plant material and biosynthesis of copper nanoparticles

The plant *Coleus aromaticus* was collected from Chennai. It was authenticated by Dr G Jeya Jothi, Assistant Professor, Dept of Plant Biology and Biotechnology, Loyola College, Chennai. After the leaves were washed and dried, it was powdered and extracted in distilled water with a soxhlet apparatus for 5 hours. The copper nanoparticles (CuNPs) were synthesized with the prepared plant extract using 0.1 M $CuSO_4$. $5H_20$ as the precursor. 70 ml of the plant aqueous extract was taken and added to 30 ml of $CuSO_4$ and kept aside for 24 hours. The solution was centrifuged at 5000 rpm for 20 mins and the pellet was dried and used for characterization.

2.2. Characterization of nanoparticles

The nanoparticles (NP) were characterized by UV-Visible spectroscopy, Fourier Transformation Infra-Red Spectroscopy (FTIR), Scanning Electron Microscopy (SEM)- Energy Dispersive Spectroscopy (EDX) and Zeta Potential to identify their absorption peak, functional groups, the size and morphology and stability.

In order to find out the wavelength of the nanoparticles, double distilled water was used to dilute the sample and the UV-Vis spectrum was recorded between the range of 200 to 800 nm. FTIR was done to find out the biomolecules present in the extract that aided in the synthesis of the NPs and the spectrum was recorded from 4000 cm^{-1} to 500 $cm^{-}1$. SEM analysis was done to study the surface morphology of the produced $CuSO_4$ nanoparticles and EDX was done to analyse the surface elemental composition. The charge that develops at the interface between a solid surface and a liquid medium is known as the zeta potential and it aids in understanding of the stability of the nanoparticles.

2.3. Evaluating the antimicrobial activity of the nanoparticles

The antimicrobial activity of the nanoparticles was examined against upper respiratory tract infection causing organisms such as *Klebsiella pneumoniae* (MTCC 4031), *Haemophilus influenzae* (MTCC 3826), *Bordetella bronchiseptica* (MTCC 6837), *Streptococcus pneumoniae* (MTCC 655), *Aspergillus niger* (MTCC 281) and *Aspergillus fumigatus* (MTCC 343) by agar well diffusion method (Durán, Nakazato, and Seabra). The samples were added in four different volumes50 μl, 100 μl, 120 μl and 150 μl. Tetracycline (30 μg) for the bacterial cultures and amphotericin B (50 μg) for the fungal cultures were used as standards. This was done in triplicates and zones of inhibition were observed after a 24-hour incubation period on the plates.

2.4. Screening for antimycobacterial activity of copper nanoparticles against the tuberculosis strain H37Rv by broth microdilution method

The antimycobacterial activity of the copper NP produced was tested against the tuberculosis strain H37Rv by broth microdilution method (Makane et al.). 0.1 ml of the test drug (the copper NP) were added in four concentrations- 1000 μg/ml, 500 μg/ml, 250 μg/ml and 125 μg/ml and its minimum inhibitory concentration (MIC) was checked. 0.1 ml of 0.1 M $CuS0_4$ was also added to a well to check if it could inhibit the growth of MTB when used separately. The experiment was carried out in duplicates. The plates were sealed and incubated for 14 days. The results were read under an inverted microscope and the inhibition of mycobacterium was determined by the absence of cord formation in the wells.

2.5. Evaluating in vitro anticancer activity of the green copper NP against A 549 lung cancer cell line and cytotoxicity studies with Vero cell line

In vitro anti- cancer assay was done to check the activity of the green synthesized copper NP against A 549 lung cancer cell line and the cytotoxicity studies were made with Vero cell line by MTT assay (Tavares-Carreón et al.). 100 μl of the test drug (copper NP) was added to each well in varying concentrations (1000 μg/ml, 500 μg/ml, 250 μg/ml, 125 μg/ml, 62.5 μg/ml, 31.25 μg/ml and 15.625

μg/ml) The experiment was done in triplicates.

The calculations were done by the following formula:

$$\% \text{ cell viability} = O.D\ of\ treated\ cell / O.D\ of\ control \times 100$$

$$\% \text{ cell inhibition} = 100 - cell\ viability$$

3. Results and discussion

3.1. Synthesis of copper nanoparticles

The aqueous solution turned brown indicating the formation of CuNP (Fig 1).

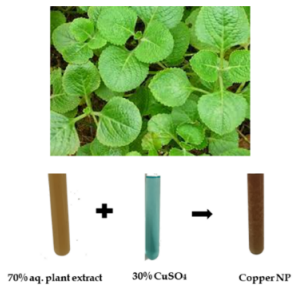

FIGURE 1. Green synthesis of copper nanoparticles

3.2. Characterization of copper nanoparticles

The UV spectrum exhibited a distinguishable peak with λ_{max} 320 nm (Fig 2) which co-related to that of copper. Previous literature indicates that the λ_{max}varied from 340 (Murthy and Ananda) to 410 nm (Pérez-Alvarez et al.).

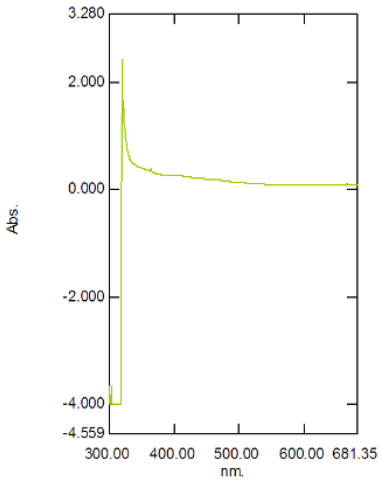

FIGURE 2. UV spectrum of copper nanoparticles depicting a peak at 320 nm

FTIR spectrum (Fig 3) revealed the presence of five peaks at 3153.32cm^{-1}, 2122.71cm^{-1}, 1578.52cm^{-1}, 1509.57cm^{-1} and 1047.38cm^{-1}. The peak at 3153 represented a medium C-H stretching alkene, 2122 was a weak C $\equiv$ C stretching alkyne, 1578 was a medium C=C cyclic alkene, 1509 was a strong N-O stretching nitro compound and 1047 was a S=O stretching sulphoxide.

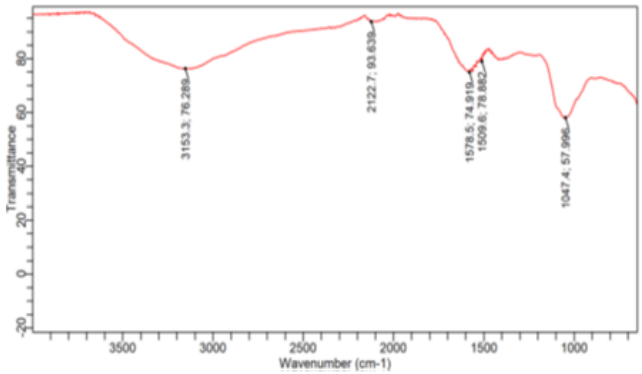

FIGURE 3. FTIR spectra of copper nanoparticles

The plant extract contains hydroxyl and carbonyl linkages that have reducing groups that chelate the metal ions which promote the nanoparticle synthesis (Mohamed).

The size and shape of the synthesized green copper nanoparticles were exhibited by SEM. They were oval and spherical in shape with varying particle size ranging from 30-50 nm (Fig 4). The EDX spectrum showed that the peaks corresponded to elemental Cu, C and O (Fig 4, Table 1). Previous studies have revealed the average particle size of green copper nanoparticles as 50-60 nm (Amjad et al.) and 5-20 nm (Mali et al.).

TABLE 1. Element and Weight %

Element	Weight %	Atomic %
C K	43.11	57.54
O K	37.49	37.56
Cu K	19.40	4.89
Totals	100.00	

The zeta potential was found to be -5.7 mV (Fig. 5). This was found to be in concordance with previous literature. The slight negative charge on the nanoparticles helps to prevent the particles from aggregating when kept in a solution for a period of time (Alhalili).

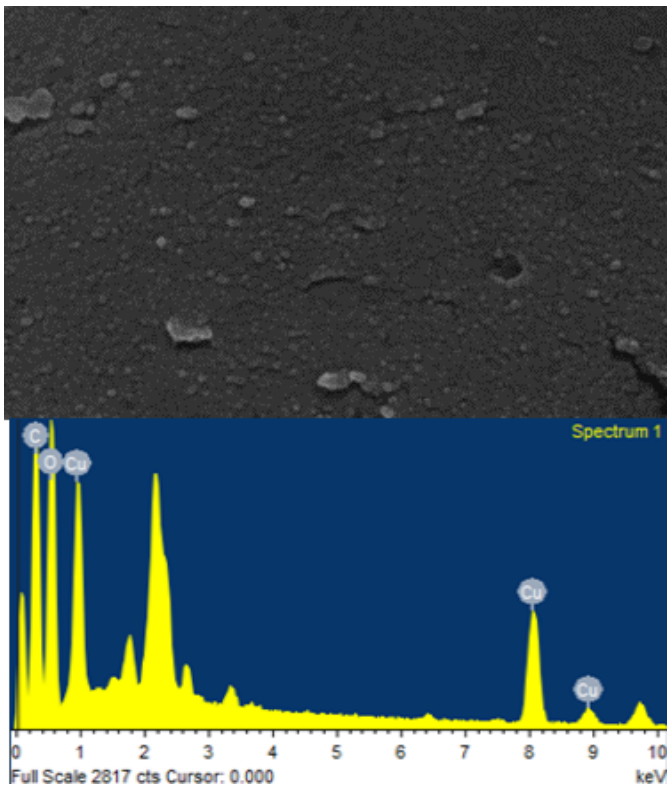

FIGURE 4. SEM and EDX visualization of the synthesized copper nanoparticles

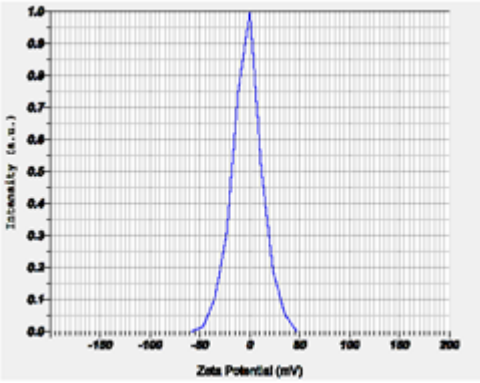

FIGURE 5. Zeta potential analysis of copper-nanoparticle

3.3. Evaluating the antimicrobial activity of the nanoparticles

The plates were checked after 24 hours and the zone of inhibition (Fig 6) was calculated in millimeter (mm) (Table 2). These zones were at par or even better than the standard antibiotic and antifungal discs.

In line with previous research, copper nanoparticles have had zones of clearance of 16 mm against *E. coli* (*Longano et al.)* 15 mm against *Staphylococcus aureus* (*Longano et al.)*and 20 mm against *Klebsiella pneumoniae* (*Wu et al.)*. No studies have been reported on *H. influenzae, B. bronchiseptica* further pressing on the novelty and the potency of the nano drug.

3.4. Screening for antimycobacterial activity of copper nanoparticles against the tuberculosis strain H37Rv by broth microdilution method

From the results in Table 3 it was observed that the positive controls showed mycobacterial growth as expected and the negative control rifampicin was able to inhibit mycobacterial growth. Amongst the test samples the copper nanoparticles at 125 and 250 μg /ml concentration were unable to prevent mycobacterial proliferation whereas the copper nanoparticles at 500 and 1000 μg /ml were able to successfully inhibit its growth. 0.1 M $CuS0_4$ when used separately was not able to inhibit mycobacterial growth. These results indicated that the MIC of the copper nanoparticles synthesized was 500 μg /ml. Previous studies indicate that gold, silver and zinc nanoparticles have been extensively used against tuberculosis. But the MIC values of Ag (Buttacavoli et al.) and Zn NPs (Qing et al.) against H37Rv strain of *M. tuberculosis* were reported to be 1.5 mg/mL and 1.25 mg/mL. The current study provides a potent and unique drug that can tackle TB at a smaller dose.

3.5. Evaluating in vitro anticancer activity of the green copper NP against A 549 lung cancer cell line and cytotoxicity studies with Vero cell line

The anticancer activity was checked using the A549 lung cancer cell line. The % cell viability and cell inhibition were calculated and found to be as follows (Table 4). These results indicated that the copper nanoparticles synthesized were very effective against lung cancer and would be potential anticancer agents. At 1000 μg/ml 92% of the cells were inhibited and even at a lower concentration of 15.625 μg/ml 69% of the cells were inhibited thus, proving to be very effective.

The cytotoxicity studies were done (Table 5) to find the toxicity levels of the sample. The results showed that nanoparticles were non-toxic with good cell viability percentage. The graph (Fig. 7) depicts the percentage viability and inhibition of the cells for both anticancer activity (A 549) and cytotoxicity (Vero).

The anti-cancer results indicated that at high con-

TABLE 2. Zone of inhibition (mm) of CuNP against upper respiratory tract infection causing organisms

Organisms	Zone of inhibition of CuNP from C. aromaticus				
	50 μl	100 μl	120 μl	150μl	Control
K. pneumoniae	20 ± 0.5	30 ± 0.61	30 ± 0.18	30 ± 0.12	10
H. influenzae	-	10 ± 0.01	10 ± 0.04	10 ± 0.10	10
B. bronchiseptica	-	-	10 ± 0.12	10 ± 0.15	12
S. pneumoniae	-	10 ± 0.12	10 ± 1.15	10 ± 2.18	12
A. niger	-	10 ± 0.19	12 ± 0.5	12 ± 0.70	-
A. fumigatus	-	10 ± 0.1	12 ± 0.16	15 ± 1.15	-

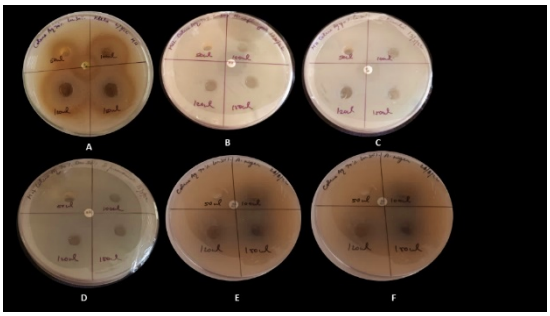

FIGURE 6. Antimicrobialactivity of the copper nanoparticles against A- *Klebsiella pneumoniae*,B- *Haemophilus influenzae*, C- *Bordetella bronchiseptica*, D- *Streptococcuspneumoniae*, E-*Aspergillusniger*, F-*Aspergillus fumigatus*

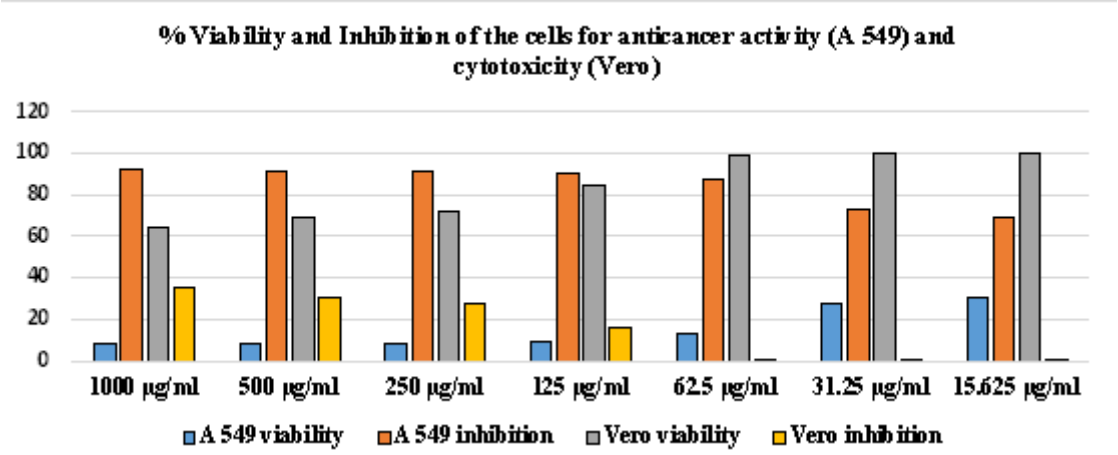

FIGURE 7. Graph depicting the percentage viability and inhibition of the cells for both anticancer and cytotoxicity activity

centration of 1000 μg/ml of sample only 7% of the cancer cells were viable and the cytotoxicity results showed that they were non-toxic since 64% of the cells were found to be viable at such high concentrations. At a lower concentration of 15.625 μg/mL only 30% of the cancer cells were viable and 100% viability was found in the case of Vero cells. Previous anticancer studies of copper nanoparticles have been made against breast cancer cell lines (Zughaibi et al.), skin cancer cell lines (Fernanda et al.), colon cancer cell lines (Gnanavel, Palanichamy, and Roopan) and cervical cancer cell lines (Chen et al.) and have found to be effective. Not much light has been thrown on the effect of green nano copper against lung cancer which is a primary cause of death globally and therefore has to be dealt with an effective drug.

TABLE 3. Antimycobacterialactivity of copper nanoparticlesagainst the tuberculosis strain H37Rv by broth microdilutionmethod

Sample	Mycobacterial growth(H37RV Standard Strain)
Control (1:10 dilution, Drug free)	Positive
Control (1:100 dilution, Drug free)	Positive
Rifampicin (1μg/ml)	Negative
0.1 M $CuSO_4$. $5H_20$	Positive
Cu E. globulus NP (125 μg /ml)	Positive
Cu E. globulus NP (250 μg /ml)	Positive
Cu E. globulus NP (500 μg /ml)	Negative
Cu E. globulus NP (1000 μg /ml)	Negative

TABLE 4. Anticancer activity of nanocopper against A549 lung cancer cell line

Concentration of sample loaded	% Cell Viability	% Cell inhibition
1000 $\mu g/mL$	7.85 $\pm$ 0.20	92.15 $\pm$ 0.20
500 μg/mL	8.23 $\pm$ 0.51	91.76 $\pm$ 0.51
250 $\mu g/mL$	8.59 $\pm$ 0.18	91.41 $\pm$ 0.18
125 $\mu g/mL$	9.24 $\pm$ 0.33	90.75 $\pm$ 0.33
62.5 μg/mL	12.68 $\pm$ 0.50	87.32$\pm$ 0.50
31.25 $\mu g/mL$	27.43 $\pm$ 3.20	72.44 $\pm$ 3.20
15.625 $\mu g/mL$	30.60 $\pm$ 0.38	69.40 $\pm$ 0.38

4. Conclusion

Green copper nanoparticles from the aqueous extract of Coleus aromaticus have the potential to inhibit tuberculosis, lung cancer and some upper respiratory tract infection causing organisms. The activity of copper nanoparticles against H. influenzae, B. bronchiseptica and S. pneumoniae were reported for the first time. In addition, the antimycobacterial activity against the H37Rv strain of tuberculosis strain proved it was effective with a MIC of 500 μg/ml. The anticancer results were also very good against the lung cancer cell line A 549. In addition, the cytotoxicity results indicated that the CuNP were not toxic to the cells. Previous studies have not been reported where the same drug is effective against two associated chronic debilitating conditions.

TABLE 5. Cytotoxicity activity of nano copper against Vero cell line

Concentration of sample loaded	% Cell viability	% Cell inhibition
1000 $\mu g/mL$	64.12 $\pm$ 3.54	35.75 $\pm$ 3.54
500 $\mu g/mL$	69.42 $\pm$ 3.49	30.43 $\pm$ 3.49
250 $\mu g/mL$	71.78 $\pm$ 4.50	27.97 $\pm$ 4.50
125 $\mu g/mL$	84.14 $\pm$ 3.06	15.68 $\pm$ 3.06
62.5 $\mu g/mL$	99.50 $\pm$ 0.70	0.95 $\pm$ 0.07
31.25 $\mu g/mL$	99.80 $\pm$ 0.28	0.35 $\pm$ 0.07
15.625 $\mu g/mL$	100.00 $\pm$ 0.01	0.01 $\pm$ 0.00

Authors' Note:

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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