GREEN SYNTHESIS OF COPPER NANOPARTICLES USING NEEM (*AZADIRACHTA INDICA*) LEAF EXTRACT AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract:

Development of eco-friendly Green synthesis of copper nanoparticles (CuNPs) is an important aspect in the field of nanotechnology and is also inexpensive method for nanoparticles biosynthesis. Recently the utilization of secondary metabolites from plant leaf extract has emerged as a novel technology for the synthesis of various nanoparticles. In the current study, copper nanoparticles were synthesized by the aqueous leaf extract of *Azadirachta indica* (Neem). 0.1 M copper sulphate solution and 25g of neem extract was prepared. 150 mL of neem extract and 50 mL of copper sulphate were taken and mixed in a beaker. It was observed that the Azardiachta indica leaf extract can reduce copper ions into copper nano particles within 5 minutes of reaction time and green to dark green coloured precipitate settling down is observed. The biosynthesized nanoparticles of copper oxide are characterized through UV-Vis spectra, FT-IR, XRD and AFM. In the present study the formation and stability of the reduced CuNPs was monitored by absorbance spectra of UV-visible spectrophotometer at different stages during the synthesis process. Metal nanoparticles in the solution were monitored by UV–Vis spectrophotometer analysis. Their peaks of CuNPs were determined with a particles size in the range of 200nm-600nm. At 350 nm UV-Vis spectra a peak was obtained due to inter band transition of core electrons and through tauc plot, band gap energy is found to be 3.64 eV. Copper nanoparticles also exhibit high antibacterial. The antimicrobial activity of the copper nanoparticles was observed by agar well diffusion method. The result indicates that the leaf extract of Azadirachta indica (Neem) shows potential antimicrobial activity at different concentrations, ensuring bioactive compounds useful in medicine

Keywords: Azadirachta indica (Neem leaf extract), Copper nanoparticles biosynthesis, characterized-UV-Vis spectra, antimicrobial activity

1. Introduction:

Nanoparticles can be synthesized by three methods, First one which includes physical method which is through evaporation, vaporization, laser ablation; while the second method is chemical in which the metal ions in solution is reduced under conditions favoring the formation of small metal oxides or aggregates. The chemical method is grouped into two classical methods one by using radiation chemicals and the other third method is biological synthesis is also a form of chemical method that are

not harmful and naturally occurring reducing agent such as plant extract or biological microorganisms such as fungi, bacteria are used. It was also reported that the metal nanoparticles formation is as the result of the antioxidant and reducing property of the phytochemicals, [2].

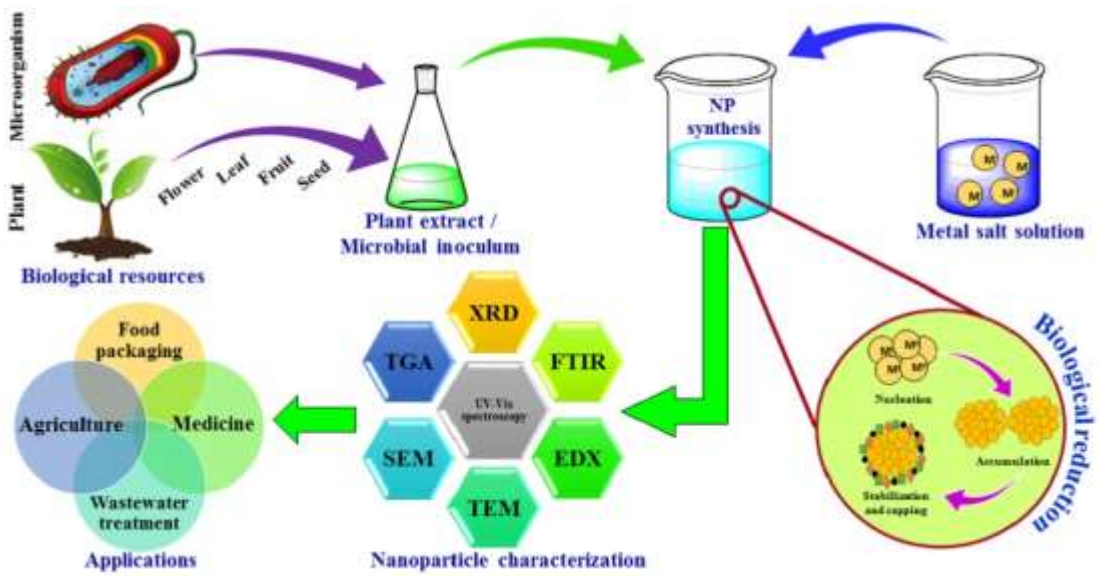

Fig.1. Synthesis of nanoparticles and Characterization.

Copper is a widely used material in the world because of various applications in electricity, optics, biomedical and catalysis. Copper nanoparticles is referred to as effective bactericidal metal because it is toxic to microorganism and non-toxic to animal cell and therefore, consider to be safe for human being in the aspect of food packaging and water treatment [3]. Due to the stability of copper nanoparticles supported on a matrix and their disinfecting properties, copper nanoparticles can be used as a bactericide agent to coat hospital equipment [2]. Iron nanoparticles are given less attention due to it extreme reactivity which has rather made it difficult to study. However, iron has very potent magnetic and catalytic property [9].

In this paper, a simple one step method for the synthesis of metal nanoparticles by the reduction of aqueous copper ions using leaf extracts of Azadirachta indica at room temperature without using any additive protecting the metal nanoparticles from aggregation. In this work the potential of copper nanoparticles was investigated, and the application of plant-based copper nanoparticles in the development of antibacterial nanoparticles was carried out by agar disc diffusion method against microorganisms[6].The production of the copper nanoparticles through bio-reduction of copper ions by the Azadirachta indica leaf extract and their antibacterial test in this work may provide valuable

technical parameters for industrialization of the biosynthetic technique and further antibacterial application of the nanoparticles[6].

The main objective of this study is to biosynthesize nanoparticles of copper using Neem (Azadirachta indica) leaf extract and evaluate their antimicrobial activity. In recent, green synthesis of Cu nanoparticles was achieved by using plant extract [2].

Plant Description:

The plant used in this work is Azadirachta indica (Neem); it is a tree in mahogany family. It is used in controlling blood sugar level and also clean blood [8]. Products from Neem are believed to be anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, Neem Plant Leaves extract is used to produce Iron contraceptive and sedative Various plant part can be used in the synthesis of metal nanoparticles i.e. the leaf, stem, root, flower and seeds [3,8].

Family Name: Meliaceae

Binomial name: Azadirachta indica

Common name: Neem

Plant part taken: Leaves

Fig.2. Neem leaf.

2. MATERIALS AND METHODS

2.1. Materials: Azadirachta indica (Neem) leaves were collected directly from the areas of Hyderabad, Telangana and the precursor $CuSo_4$ were obtained from Sigma Aldrich.

2.2. Preparation of extract: The extract was prepared by collecting the selected Azadirachta indica (Neem) and washed in running tap water and then rinsed with distilled water for the removal of

impurities. 100g of leaves were chopped into small pieces with a sterile knife and were grinded using motor and pestle and was then boiled for 10 min at 60° C and filtered using Whatman's no. 1 filter paper. The filtrate was collected in clean, dry 250ml conical flask and stored in refrigerator for further use.

2.3. Preparation of 0.1 M Copper Sulphate Solution

About 2.49 g of copper Sulphate salt was weighed accurately and made upto 250 mL in a Standard flask and stored [8].

Fig.3. Neem leaf Extract & 0.1M $CuSo_4$

2.4.Preparation for Copper Nanoparticles (CuNPs): In the synthesis of copper nanoparticles, the crude plant extract is mixed with the aqueous solution of $CuSo_4$ in 3:1 proportions [8]. The crude Neem leaf extract 150ml is mixed with 50ml 0.1M $CuSo_4$ solution and solution was then allowed for a day in dark place. The change in colour of the mixture from green to dark green within few minutes indicates the formation of metal nanoparticles.

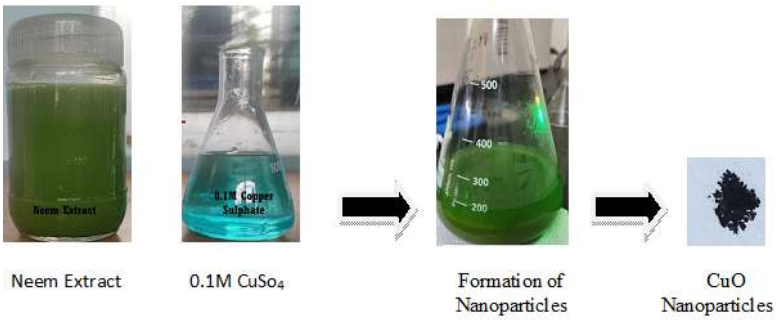

Fig.4.Synthesis of CuO Nanoparticles.

2.5.Characterization of CuNPs: The synthesized metal nanoparticles were centrifuge for 10min at 5000rpm. The sediment was then collected with deionized water and kept for SEM and FTIR analysis.[6]. The UV-Vis spectroscopy measurement was recorded at the wavelength of 200nm, 250nm, 300nm, 350nm, 400nm, 450nm, 500nm, 550nm 600nm [3].Then the metal nanoparticles which is transferred into the cuvette for analysis at various wavelengths and absorbance were recorded.

2.6. Antimicrobial Activity of copper nanoparticles:

The antimicrobial activity of the metal nanoparticles was observed by well diffusion method [5]. Luria Bertini Agar media is used for our study.

3. Result and Discussion:

3.1. UV-Visible Spectrophotometric Analysis:

UV-Visible Spectrophotometric Analysis of CuO NPs was done and repeated several times, in the wavelength range of 200-600 nm and the optical densities was recorded as given in the table-1. A peak was obtained at **350 nm** due to inter band transition of core electrons of CuO NP and the spectrum was represented in Fig.5. The absorbance wave length values are closely matched with the reported values.

S.no	Wave Length(nm)	O.D
1.	200	3.004
2.	250	3
3.	300	2.899
4.	350	3.012
5.	400	2.85
6.	450	2.78
7.	500	2.099
8.	550	2.042
9.	600	2.027

Table.1. O.D Values at wavelengths 200-600 nm.

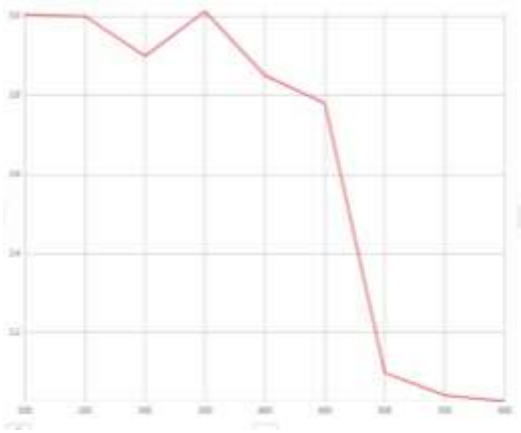

Fig. 5. UV-Vis spectra of CuO nanoparticles.

3.2.Tauc plots is used to determine the band gap energy. To study the optical absorption spectrum of a material Tauc plots are very convenient method. The absorption coefficient α for direct band gap material from the Tauc relation,is given by,

$\alpha h\nu = A(h\nu - Eg)m$

where α is the absorption coefficient, A is the optical constant, Eg is the optical band gap and m is an index which assumes the values 1/2, 3/2, 2 and 3 depends on the nature of electronic transition which is responsible for the reflection.

Tauc plot was used for determining the band gap energy. Tauc plot has the photon energy (hν) on the X axis and a quantity (αhν)2 on the Y axis and extrapolating the linear portion of the curve on the X-axis which yields the band gap energy of the material. The band gap energy of CuO Nanoparticles is found to be 3.64 eV (Fig. 6).

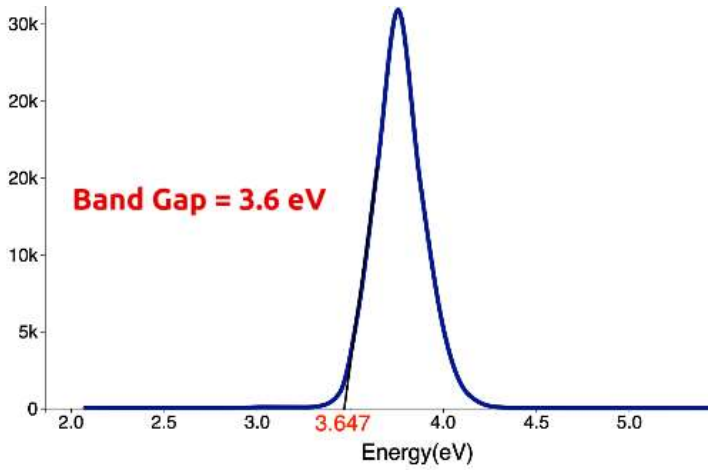

Fig. 6 Tauc plots of CuO nanoparticles

3.3. Antimicrobial Activity of copper nanoparticles:

The antimicrobial activity of the metal nanoparticles was observed by well diffusion method [5]. Luria Bertini Agar media is used for our study. Sterilized media was transferred into clean, dry and sterilized Petri dishes then allowed to cool to solidify. Later upon solidification, the bacterial cultures were spread on the agar surface in the Petri dishes. By using a sterile hole borer, hole was made in the media and then filled with various concentration of synthesized nanoparticles [6]. Then the Petri-dishes were incubated in an incubator for 24hrs at 37^0 C. After incubation, as the concentration of synthesized CuO nanoparticles increased, the diameter and growth inhibition zones are also gradually increase and were recorded.

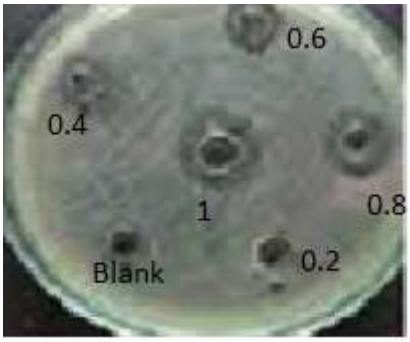

Fig. 7. Antimicrobial activity of the CuO nanoparticles

4. Conclusion:

In conclusion, the development of eco-friendly Green synthesis of copper nanoparticles (CuNPs) is an important aspect in the field of nanotechnology and is also easily available starting materials, inexpensive method for nanoparticles biosynthesis and also easy to carry out in any laboratory, use of toxic reagent is avoided and pollution free, From our Study we report eco-friendly synthesis of CuO NPs using a leaf extract of Azadirachta indica (Neem) and $CuSo_4$. UV-Visible Spectrophotometric Analysis of CuO NPs was done in the wavelength range of 200-600 nm. A peak was obtained at 350 nm due to

inter band transition of core electrons of CuO NP and The band gap energy of CuO nanoparticles is found to be 3.64 eV from the Tauc plots. The antimicrobial activity of the copper nanoparticles was observed by agar well diffusion method. The result indicates that the leaf extract of Azadirachta indica (Neem) shows potential antimicrobial activity at different concentrations ensuring bioactive compounds useful in medicine. Further investigation is needed to understanding the mechanism of action as an antimicrobial agent.

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