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Synthesis of Copper Nanoparticles via *Trigonella Foenum-Graecum* Seed Extract for Antibacterial Response

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Abstract

In view of the immense capability of plants this work is planned to employ seed extract as a source for the reduction of Cu ions in to Cu nanoparticles (Cu NPs). For this purpose seed extract of *Trigonella foenum-graecum* (fenugreek seeds) was utilized as a substitute of classical methods. This green path for synthesizing Cu NPS is easy, natural, low cost, sustainable and eco-friendly as compared to conventional methods. In this experiment harmful chemical/physical methods for the production of Cu nanoparticles is replaced by using minimum concentration of seed extract. The stepwise characterization was done by using Atomic Absorption spectroscopy, UV-Vis Spectroscopy, FTIR spectrophotometer and X-ray diffraction (XRD) which have given much valuable information about these materials. Antibacterial activity of these nanoparticles is observed at different concentration so their ZOI (Zone of inhibition) and MIC (minimum inhibitory Concentration) was also calculated against four human pathogenic strains.

Keywords: Nanoparticles, Synthesis, Seed extract, FTIR, XRD, Bacterial culture.

Introduction

Nanotechnology has achieved a prominent place in today's research. With reducing size (1-100 nm) nanoparticles possess a higher surface to volume ratio which is significant for catalytic reactivity and other similar properties such as antimicrobial activity in nanoparticles [1]. Nanoparticles can be prepared by either physical methods including pulsed laser ablation [2], vacuum vapor deposition [3], pulsed wire discharge [4], mechanical milling [5] and chemical methods involving chemical reduction [6], micro emulsion techniques [7], sonochemical reduction [8], electrochemical [9], microwave assisted [10] and hydrothermal methods [11].

he usage of lethal chemicals and by products with in these methods make the nanoparticles synthesis more challenging and problematic. Moreover, these methods also demand high energy and uneconomical purifications. Green synthesis provides advancement over both chemical and physical methods as it is economical, eco-friendly, capable for large scale synthesis and does not require consumption of harmful chemicals, high energy, temperature and elevated pressure [12]. Presently green chemistry involve the biosynthesis through microorganism and plant extracts [13, 14]. By using natural extract of *Aspalathus Linearis* crystalline perovskite $ZnSnO_3$ nanoclusters, NiO, Pd & pdO nanoparticles are biosynthesized [15,16]. Sageretia thea & *Moringa Oleifera* natural extracts are used as chelating agent to prepare ZnO nanoparticle [17,18].

Copper nanoparticles exist in three forms i.e. Cu metal nanoparticle, Cu(I) oxide nanoparticle [Cu_2O] and Cu(II) oxide nanoparticle [CuO].These have catalytic [19], optical [20] & sensing activities [21]. They are also helpful as resistance materials [22], inorganic-organic nanocomposite [23] & in solar cells [24]. Metal

nanoparticles can show a good antibacterial response. This antibacterial activity of Cu may be accredited to their micron range size less than the pore size of the bacteria and thus, they are capable of easily crossing the cell membrane without any hindrance [25].

Fenugreek, *Trigonella foenum-graecum* (Greek hay) is an annual herb of the pea family (*Fabaceae*) cultivated in North Africa, the Middle East and Asia. It is used mostly as ingredient in traditional medicine in Egypt, India and Asia. Fenugreek helps to heal inflammation and its extracts can be added in manufacturing cosmetics and also in soaps due to its long history as anti-tumorigenic, antioxidant, antidiabetic, and antimicrobial activities. Its seeds Extract help control in digestion, arthritis, diabetes, cholesterol, cancer, heart attack, improvement in breast milk, kidney and liver function [26, 27].

his research work aims to throw light on the bio reduction of Cu^{+2} ions into Cu^{0} nanoparticles by employing aqueous Fenugreek seeds extract under varied percentage compositions and there by implementing eco-friendly chemistry for the future research. To the best of our knowledge this green synthesis of Copper nanoparticles by using aqueous extract of Fenugreek seeds is a novel research and has not been reported earlier.

Experimental

Addition of the aqueous plant extract into a solution of the appropriate metal salt initiates a plant extract-facilitated green reduction. The preparation of NPs takes place at room temperature and completes within a few minutes.

Seed extract preparation

They were thoroughly washed with distilled water, dried and crushed. The powder was further used for preparation of 1 g/100 mL aqueous seeds extract. This extract was boiled for 30 minutes approx. at 80-90ºC, shaked (5 c/s for 10 minutes) & filtered to obtain homogenized solution which was kept at 4°C in 250 mL Erlenmeyer flask. In each and every step of the experiment, sterility conditions were maintained for the efficacy and accuracy in results without any impurity.

Biosynthesis of nanoparticles

For biosynthesis of nanoparticles, 2.0 mL of the seeds extract was mixed with 20 µL of freshly prepared 1×10^{-2} M aqueous copper sulphate pentahydrate solution in 250 mL Erlenmeyer flask under continuous magnetic stirring. The Cu nanoparticles obtained were purified by repeated centrifugation method at 6,000rpm for 40 min followed by dispersion of the pellet in deionized water. Later the Cu nanoparticles were dried in an oven at 90-100 ºC for 3-4 hours [28].

Table 1 shows the preparation of different concentrations by varying the amount of seed extract and keeping the concentration of $CuSO_4.5H_2O$ constant i.e., 1×10^{-2} M.

Table 1. **Procedure for the preparation of different concentrations**

Concentration	Procedure
5%	5mL of the seed extract (Fenugreek) was mixed with 95mL of the 0.01M $CuSO_4$ Solution.
15%	15mL of the seed extract was mixed with 85mL of the 0.01M CuSO4 Solution.
25%	25mL of the seed extract was mixed with 75mL of the 0.01M CuSO4 Solution.

Procedure for Antibacterial Activity

For antibacterial activity agar well diffusion method was employed [25]. In each well 50uL of the sample was dispensed . Four bacterial strains were selected including *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 7002), *Agrobacterium tumefaciens* (ATCC 5577) *and Bacillus subtilis* (ATCC 6051). For each strain different concentrations 5%, 15% & 25% was prepared and also measured the zone of inhibition (ZOI) of each in mm [29].

Biosynthesized nanoparticles characterization

1mL of the colloidal solution of prepared Cu NPS was diluted with 10 mL of the distilled water and then this diluted solution was aspirated

into the atomic absorption spectrophotometer through capillary tube to confirm the NPs synthesis. UV-Vis spectral analysis was also done between 200-700 nm. FTIR spectral studies was done on Shimadzu IRTracer-100, fourier transform infrared spectrometer between 4000 and 375 cm^{-1} with resolution of 4 cm^{-1}. The powder X-ray diffraction was conducted on a Philips Analytical XPERT diffractometer using a Cu Kα radiation (λ = 1.540598 °A) with a MINIPROP detector and operating at 40 kV generator voltage and 40 mA generator current. X-ray diffraction patterns were recorded between 2θ = 5◦ and 79◦ with a step of 0.04◦ and a time of 0.2 s by step.

Results and Discussion

Atomic absorption spectrometry

The presence of copper nanoparticles was confirmed by concentration-absorption linear relationship. Fig. 1 shows the increase in absorption with the growing concentration of Cu nanoparticles with gradual increase of seed extract concentration.

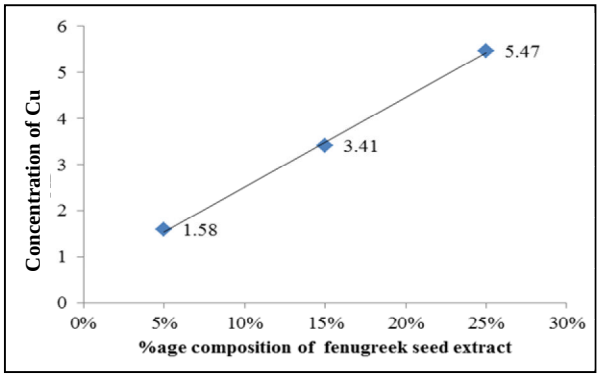


Figure 1. **Effect of the Fenugreek seed extract percentage composition on the synthesis of Cu NPs.**

Visual observation and UV-Vis spectroscopy

Reduction of Cu^{+2} into Cu^{0} during addition of fenugreek seeds extract is monitored as a consequence of the change in colour and thus can be studied through UV-Vis spectroscopy. The change in colour is because of the Surface Plasmon Resonance (SPR). The SPR band was observed at a maximum absorbance of approximately 560 nm which was confirmed from the literature value [30].

Different parameters were optimized including concentration of seeds extract and reaction time. It was identified that the yields of copper nanoparticles were affected by varying these parameters.

Effect of contact time at room temperature

Increasing the reaction time results in gradual increase of absorbance which was quantitatively monitored (Fig. 2). Furthermore, the color intensity also increases with the duration of incubation from faint blue to yellow brown and then to deep brown as shown in (Fig. 3). This increase in absorbance and color intensity is attributed to the higher concentration of Cu NPs. [31].

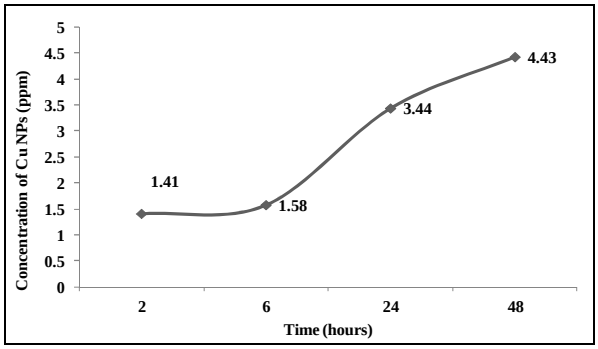
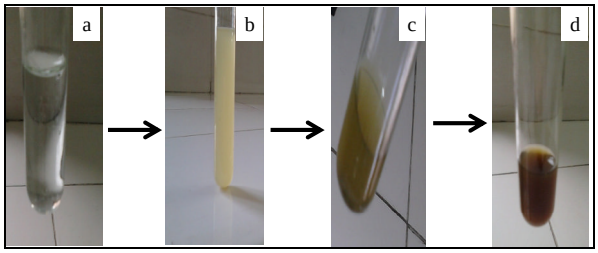


Figure 2. **Cu NPs concentration at different time intervals with 15% seed extract added to 0.01M $CuSO_4$ Solution**



A: 0.01M $CuSO_4$ Solution
B: Aqueous seed extract
C: Addition of seeds extract into 0.01M $CuSO_4$ Solution
D: Nanoparticles emulsion after 24 hrs.

Figure 3. **Formation of Nps with colour change.**

Increase in absorption intensity

As the concentration of the fenugreek seeds extract increases, the absorption peak gets more sharpness with slight increase in wavelength. If we increase the extract concentration from 5% to 25%, there is

increase in intensity of absorption as presented in Fig. 4.

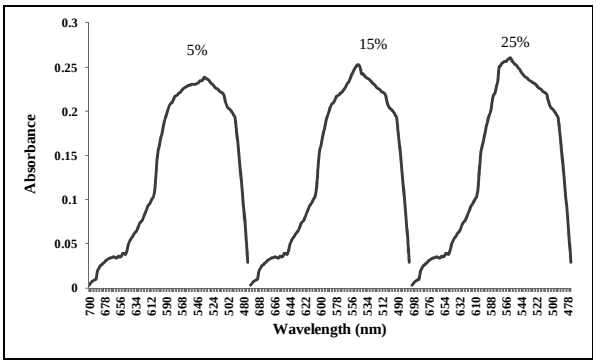


Figure 4. **For 5% seed extract absorbance is observed at 536nm and with 25% at 560 nm**

Fourier transform infrared spectroscopy (FTIR)

Fig. 5 shows FTIR spectrum of Cu nanoparticles from Fenugreek seeds extract. A wide band at 3358 cm^{-1} is due to the OH group indicating the presence of intermolecular hydrogen bonding. A peak at 2358 cm^{-1} is observed which can be attributed to C≡C stretching vibrations i.e. alkyne group present in phyto constituents of extract. A band at about 1645 cm^{-1} is due to C=O stretching vibrations associated with amide I functional group. The amide I band is directly linked to the backbone conformation indicating the presence of proteins in seeds extract [32].

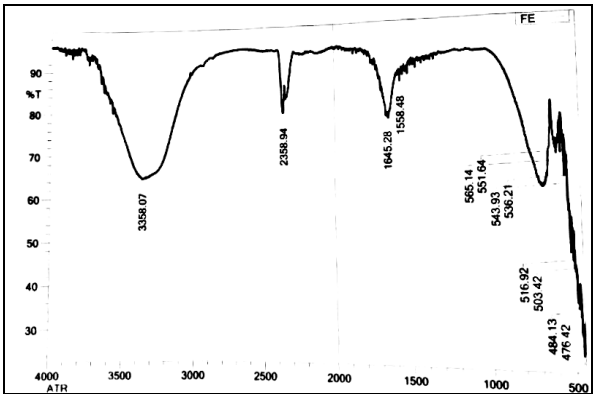


Figure 5. **FTIR spectrum of Cu nanoparticles synthesized from Fenugreek seeds extract**

X- Ray diffraction studies

The results of XRD pattern analysis revealed three intense peaks in the whole spectrum of 2 theta values ranging from 20 to 80 for the Cu nanoparticles. The sample demonstrated a high crystallinity level with diffraction angles of 28.86, 32.14 and 46.55 which correspond to the characteristic of face-centred cubic of copper lines indexed at (210), (111), and (222) Fig. 6. Peaks for Cu Nps are compared with JCPDS literature. Cu nanoparticles with different sizes were obtained. By applying Debye–Scherrer equation to the obtained XRD pattern of the Cu NPs, the average nanoparticles size was found to be 14-17 nm, which may indicate a high surface area-to-volume ratio of nanocrystals.Cu nanoparticles are easily prone to oxidation and results in the formation of CuO and Cu_2O nanoparticles as shown below [33].

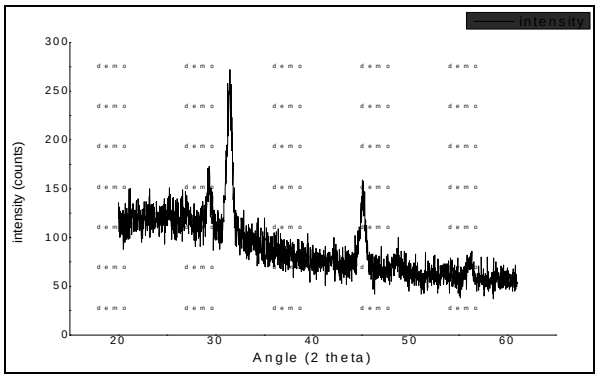


Figure 6. **XRD plot of Cu NPs synthesized from fenugreek seeds extract**

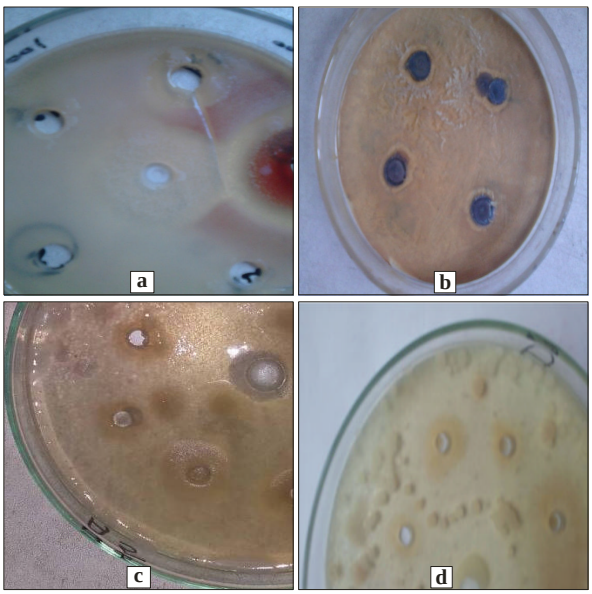
$$Cu^{+2} + 2e- \rightarrow Cu^{0} + Air\ (O_2) \rightarrow CuO/\ Cu_2O$$

But by using fenugreek seed extract as reductant Cu NPs were not oxidized to CuO NPs. Thus, from these observations it may be concluded that fenugreek seed extract is not only an excellent reducing agent but also a strong capping agent that it coats the surface of Cu NPs avoiding its contact with the air and hence no oxidation took place over the surface of synthesized NPs.

Determination of MIC (minimum inhibitory concentration)

Four bacterial cultures including *Bacillus subtilis* (one Gram positive), *Pseudomonas aeruginosa, Proteus mirabilis, Agrobacterium tumefaciens(*three Gram negative bacteria) were

used to check the antibacterial potential of Cu nanoparticles (5% to 25% concentration) synthesized from fenugreek seed extract. Ciprofloxacin was used as control against Gram positive bacterium while amoxicillin for Gram negative bacteria. 25% (Max) concentration give maximum antibacterial potential i.e., 10 mm zone of inhibition for *Bacillus subtilis*, 5 mm for *Pseudomonas aeruginosa*, 9 mm for *Proteus mirabilis* and 10 mm for *Agrobacterium tumefaciens* Table 2. With 5% concentration which is Minimum Inhibitary Concentration (MIC) 5 mm ZOI was observed for *Bacillus subtilis*, 2 mm for *Pseudomonas aeruginosa*, 3 mm for *Proteus mirabilis* and 5 mm for *Agrobacterium tumefaciens* Fig. 7.



A = Bacillus subtilis **B** = Pseudomonas aeruginosa
C = Proteus mirabilis **D** = Agrobacterium tumefaciens.

Figure 7. Observed antibacterial activities against four human pathogenic strains

Conclusion

In this research a simple, convenient, cost effective, significant and eco-friendly method for the synthesis of copper nanoparticles through the reduction of copper salts has been demonstrated. Aqueous seed extract of fenugreek is used as reducing and capping agent. Characterization of Cu nanoparticles was done by AAS, UV spectroscopy, FTIR spectroscopy and XRD analysis. These nanoparticles were evaluated for their antibacterial activity against one gram positive and three gram negative bacterial strains. These biologically synthesized copper nanoparticles using seed extract showed sufficient antibacterial potential. The minimum inhibitory concentration of nanoparticles synthesized from aqueous seed extract of fenugreek was 5%. In the concluding remarks, the copper nanoparticles synthesized using fenugreek seed extract would be a better antimicrobial effective against various bacterial species.

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