ANTIBACTERIAL ACTIVITY OF Cu_2O NANOPARTICLES ON E.COLI SYNTHESIZED FROM TRIDAX PROCUMBENS LEAF EXTRACT AND SURFACE COATING WITH POLYANILINE

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The exploitation of various plant materials for the biosynthesis of nanoparticles is considered a green technology because it does not involve any harmful chemicals. The present study reports the synthesis of Cu_2O nanoparticles from Fehling's solution using *Tridax procumbens* leaf extract. Water-soluble carbohydrates present in the plant materials were mainly responsible for the reduction of copper ions to nano-sized Cu_2O particles. The oxidation resistances of Cu_2O nanoparticles were improved by surface coating with polyaniline by chemical polymerization method using hydrogen peroxide as oxidizing agent. By taking Escherichia coli as a model for Gram-negative bacteria, which always causes a variety of suppurative infections and toxinoses in humans, as a model bioparticle, the negative bioeffect of nano-Cu_2O on E.coli cells was evaluated by disc diffusion method. The resulting Cu_2O nanoparticles and PANI coated Cu_2O were characterized by X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), UV-Visible absorption and Fourier-transform infrared (FTIR) spectroscopy.

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1. Introduction

In the fields of nanoscience and nanotechnology, the largest activity has been focused on the synthesis of new nanoparticles with different sizes and new shapes, which have strong effects on their widely varying properties. Nanoparticles are attracting increasing attention on account of their potential applications and unique properties, which are strongly influenced by their size, morphology and structure. In recent years, much attention has been paid to metal oxide nanoparticles that give rise to unique electronic and optical properties that are useful for a variety of new technologies in optoelectronic devices, chemical sensors, molecular catalysts and magnetic materials.

Metal oxide nanoparticles (NPs) have shown great potential in the field of sensing, optoelectronics, catalysis, solar cells and so on, due to their physical and chemical properties different from those in the bulk material. Among all the metal oxides, copper oxide nanomaterials have attracted more attention due to their unique properties. Cuprous oxide (Cu_2O) is a p-type semiconductor with a direct band gap of 2.17 eV [1]. In recent years, there is a growing interest to synthesize Cu_2O nanostructures not only for the development of synthetic strategies, but also for the examination of their sensing, catalytic, electrical and surface properties.

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Cu_2O nanostructures have been demonstrated to possess properties useful for applications in gas sensing[2], CO oxidation[3], Photo catalysis[4-8], photochemical evolution of H_2 from water[9], photocurrent generation[10, 11] and organic synthesis[12, 13]. Cu_2O has been prepared by several different methods, such as electro deposition [14-16], sonochemical method [17], thermal relaxation [18], liquid phase reduction [19] and vacuum evaporation [20].

Green chemistry is the design, development and implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and the environment. In synthesis of metal oxide nanoparticles by reduction of the corresponding metal ions, there are three areas of opportunity to engage in green chemistry:

(i) Choice of solvent (ii) the reducing agent employed, and (iii) the capping agent used. The *Tridax procumbens* leaves posses biomolecules such as carbohydrates, proteins and lipids[21], which could be used as reductants to react with copper ions and as scaffolds to direct the formation of Cu_2O NPs in solution. In our previous work has been reported about the synthesis of Cu_2O nanoparticles by green method [22].

Conducting polymers have emerged as a very important class of materials because of their unique electrical, optical and chemical properties leading to a wide range of technological applications. Among various conducting polymers, polyaniline is a unique and promising candidate for practical applications because of its facile synthesis [23], stable chemical and environmental properties [24]. Until now Polyaniline has been successfully coated on many materials such as Cu, Ni, Al, Pt, Fe, Zn etc. To improve the oxidation resistance of cuprous oxide, chemical polymerization method was used to coat cuprous oxide with polyaniline.

Qingyi Lu *et. al* [25] reported a one step protocol for synthesis of Cu_2O nanoparticles. They found high antimicrobial and bactericidal activity of Cu_2O nanoparticles on Gram-positive and Gram-negative bacterias. Recently, Huang and co-workers demonstrated that Cu_2O nanoparticles exhibit excellent biocidal action against *Staphylococcus aureus* bacteria [26]. Our aim is to investigate the antibacterial activity of Cu_2O nanoparticles on E.coli synthesized from *Tridax procumbens* leaf extract.

2. Experimental details

2.1 Materials

All the chemicals, $CuSO_4.5H_2O$, potassium sodium tartarate tetra hydrate and NaOH, aniline and hydrogen peroxide were of analytical grade and were used as received from Merck without further purification. E. coli strain MTCC 1687 (ATCC 8739) was purchased from Institute of microbial Technology (Chandigarh, India). The components of the Luria–Bertani (LB) medium used in growing and maintaining the bacterial cultures were supplied by Projen Laboratories (Chennai, India).

2.2 Preparation of Fehling's solution

Fehling's solution which is comprised of equal parts of the following solutions was first made:

Solution 1: Was made by dissolving of copper (II) sulfate penta hydrate (6.9 g 0.02 mol) in distilled water (100 mL).

Solution 2: Was made by dissolving of potassium sodium tartarate tetrahydrate (34.6 g) and sodium hydroxide (12 g) in distilled water (100 mL).

2.3 Preparation of *Tridax procumbens* leaf extract

About 20g of freshly, taxonomically authenticated healthy leaves of *Tridax procumbens* were collected, washed thoroughly with double distilled water, cut into fine pieces and boiled with 100mL double distilled water in Erlenmeyer flask for 8-10 min. The extract was cooled to room temperature and filtered through whattman filter paper (no.42).

2.4 Preparation of Cu_2O Nanoparticles

In a typical experiment, 10mL of the *Tridax procumbens* leaf extract was added to 10 ml of a Fehling's solution. After 10 minutes, the colour of the solution changed from blue to brick-red, indicating the formation of cuprous oxide nanoparticles. The product was washed with distilled water and then dried at room temperature.

2.5 Coating of copper (I) oxide with Polyaniline

In the typical experiment, 2.7g aniline was dissolved into 300ml deionized water and then 1.08g Cu_2O was added into this solution and followed by 68ml of 6% H_2O_2 was added slowly within 0.5h at room temperature. The reaction was carried out for 23h at constant stirring rate. The final product was filtered, washed with distilled water and then dried.

2.6 UV-Vis spectral analysis

UV-VIS spectra were measured using a TU-1901 model UV- Visible double beam spectrophotometer (Beijing Purkinje General Instrument Co., Lt, China).

2.7 FTIR spectral analysis

FTIR spectra were performed and recorded with a Fourier-transform infrared spectrophotometer Nicolet 870 between 4000 and 400 cm^{-1}, with a resolution of 4 cm^{-1}.

2.8 SEM analysis

The morphologies and compositions of the Cu_2O nanoparticles were examined by Scanning Electron Microscopy (SEM), using a LEO 1455 VP equipped with energy dispersive.

2.9 X-ray diffraction studies

X-Ray Diffraction (XRD) patterns were recorded with a Philips analytical X-ray diffractometer Using CuKα radiation (λ= 1.5406 Å).

2.10 Antibacterial activity tests

The antibacterial activity of Cu_2O nanoparticles was evaluated against Escherichia coli as a model for Gram-negative bacteria by disc diffusion method. Approximately 10^5 colony-forming units (CFU) of E.coli were inoculated on agar plates and then using a micropipette, different concentrations of the sample of nanoparticles solution (10μl, 20μl and 50μl) was poured onto each well on all plates. Cu_2O -free agar plates cultured under the same conditions were used as a control. The plates were incubated for 24 hours at 37^0C and the numbers of colonies were counted. The counts on the three plates corresponding to a particular sample were averaged.

3. Results and discussion

The addition of Fehling's solution to the leaf extract containing carbohydrates as a major component, which have aldehyde groups, may cause the reduction of Copper ions which results in the formation of brick red Cu_2O precipitate. The chemical reaction which occurs can be seen in the following equation:

$$RCHO + 2Cu^{2+} + 2H_2O \longrightarrow RCOOH + Cu_2O + 4H^+$$

To improve the oxidation resistance of fine Cu_2O powder, a new catalytic oxidation method was used to coat Cu_2O nanoparticles with polyaniline. In the method, some Cu_2O on the surface of Cu_2O particles was converted into Cu^{2+}, and with the catalysis of Cu^{2+} aniline was oxidized by hydrogen peroxide to form the polyaniline coating layer on Cu_2O particles. H_2O_2 is the main oxidation agent which will oxidize aniline into polyaniline with the catalysis of Cu^{2+} [27].

Fig. 1 shows UV-Visible spectra of Cu_2O nanoparticles and PANI coated Cu_2O. The peak at 260nm is due to inter band transition of core electrons of copper and copper oxide shown in Fig.1 (a) [22]. The absorbance peak at 382nm was due to the formation of copper (II) complex of aniline shown in Fig.1 (b) [28].

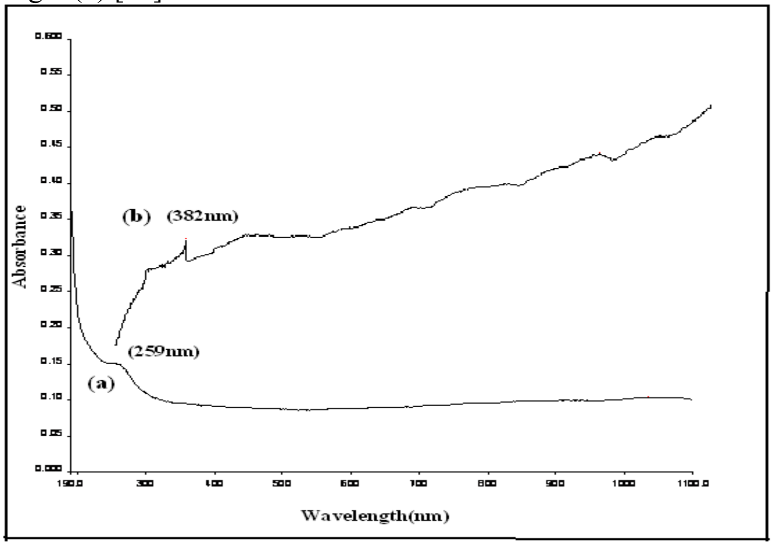

Fig 1. UV-Vis spectrum of (a) Cu_2O nanoparticles and (b) Polyaniline coated Cu_2O nanoparticles. Figure 2 represents FT-IR spectrum of Cu_2O nanoparticles synthesized by using

Figure.2 (a&b) represents FT-IR spectrum of Cu_2O nanoparticles synthesized by using *Tridax procumbens* leaf extract and PANI coated Cu_2O.. The absorption peaks in Fig 2 (a) are located mainly at 3444cm^{-1}, 1644cm^{-1}, 1337cm^{-1} and 618cm^{-1} in the region 500cm^{-1}-4000cm^{-1}. The peak at 3444cm^{-1} is the characteristic band of hydrogen bonded OH groups present in the aqueous phase. The peaks at 1644cm^{-1} (asymmetric) and 1337cm^{-1}(symmetric) indicate the presence of (-COO-) carboxylate ions, responsible for stabilizing the Cu_2O nanoparticles. The peak at 618cm^{-1} indicates the Cu (I)-O vibration of Cu_2O nanoparticles [29]. In Fig 2 (b) the peaks at 3273-3235 cm^{-1} are due to N-H vibration and deformation. The ring stretching of N=Q=N (Q denotes quinine ring) and N-B-N (B denotes benzene ring) bands appear at 1570 and 1496 cm^{-1} respectively. The peaks at 1288 and 1146 cm^{-1} correspond to C_{ar}–N and C-N stretching respectively. The peak 2960-2990 cm^{-1} can be assigned to the asymmetric and symmetric C-H stretching of PANI [27]. The peak at 682cm^{-1} indicates the Cu (I)-O vibration of Cu_2O nanoparticles in PANI coated Cu_2O.

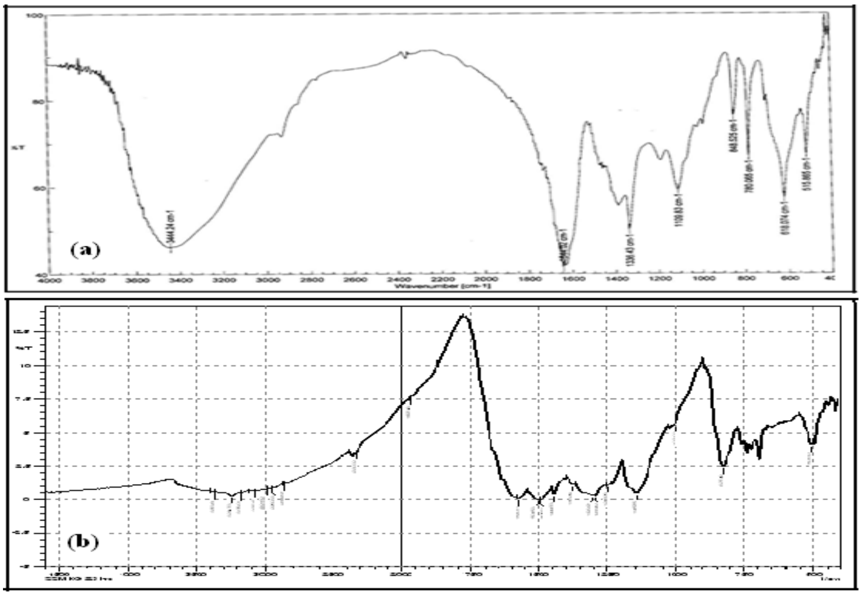

Fig 2. FT-IR spectrum of (a) Cu_2O nanoparticles and (b) Polyaniline coated Cu_2O nanoparticles.

Fig. 3 shows the XRD patterns of Cu_2O nanoparticles synthesized by using *Tridax procumbens* leaf extract and PANI coated Cu_2O. The peaks with 2θ values of 29.36°, 36.29°,43.69° and 61.78° correspond to the crystal planes of (110), (111), (200) and (220) respectively, of crystalline Cu_2O. The crystallite sizes can be estimated using Scherrer's formula $D = K\lambda/\beta\cos\theta$ where the constant K is taken to be 0.94, λ is the wavelength of X-ray , β and θ are the half width of the peak and half of the Bragg angle respectively. Using the equation, the crystallite sizes were found to be in the range of 60-80nm. The peaks with 2θ values 25.12° and 26.76° confirmed that PANI coated Cu_2O exhibit crystalline state [30].

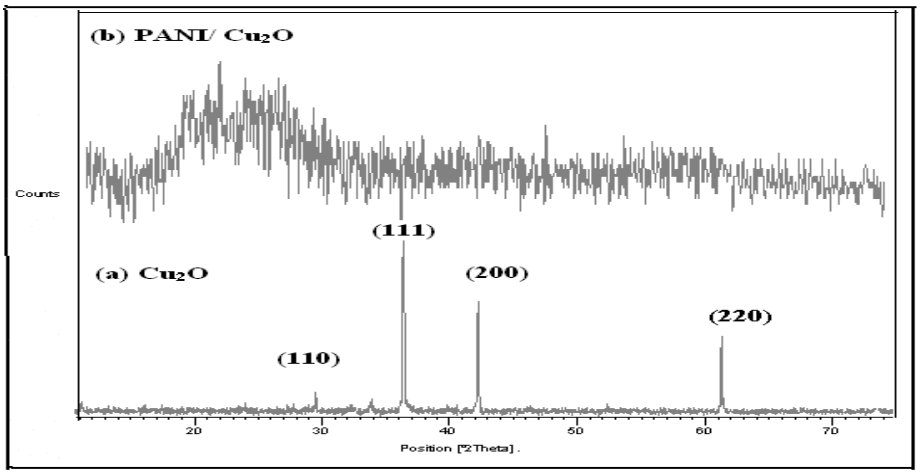

Fig. 3. XRD patterns of (a) Cu_2O nanoparticles and (b) Polyaniline coated Cu_2O nanoparticles.

In order to investigate the morphologies of the synthesized Cu_2O nanoparticles and the resultant PANI coated Cu_2O, they were characterized by scanning electron microscopy. The SEM images (Fig 4 a,b) show that the shapes of the Cu_2O nanoparticles appear like hexagonal and cubic with rough surfaces. The SEM image of PANI coated Cu_2O (Fig 4d) shows that the particles have core-shell structure and their surface was coated with a platelet like polyaniline.

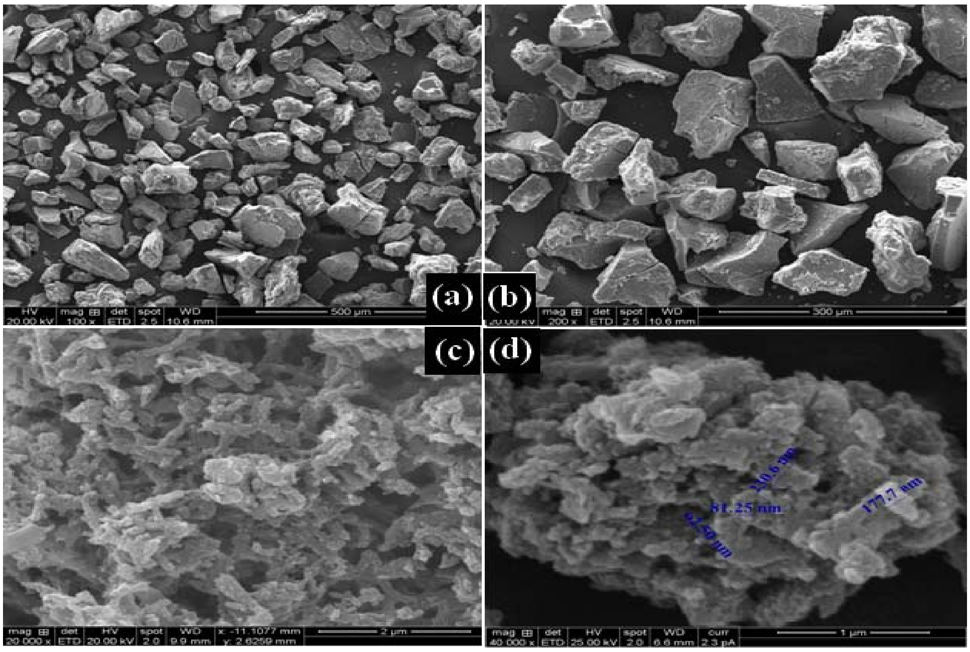

Fig 4. SEM micrographs of (a), (b) Cu_2O nanoparticles, (c) Polyaniline and (d) Polyaniline coated Cu_2O nanoparticles.

Antibacterial tests were performed against the Gram-negative bacterium E. coli on LB agar plates containing different concentrations of nanoparticles. Fig. 5 shows the number of bacterial colonies grown on LB plates as a function of the concentration of Cu_2O nanoparticles when approximately 10^5 CFU were applied to the plates. The presence of these particles at a concentration of 20 μg cm^{-3} inhibited bacterial growth by 65%. The size of the bacterial colonies grown on plates with more than 30 μg cm^{-3} of nanoparticles was significantly reduced and the colonies were mostly located at the edges of the agar plates. A concentration of 50–60 μg cm^{-3} caused 100% inhibition of bacterial growth.

The mechanism of inhibitory action of Cu_2O nanoparticles on microorganisms is partially known. The possible antibacterial mechanism is the following: Cu_2O nanoparticles are absorbed on cell surface, they impair the cell wall and then damage the cell membrane, increasing the permeability of cell membrane and leading to a decrease in the viability of bacteria in Cu_2O solution.

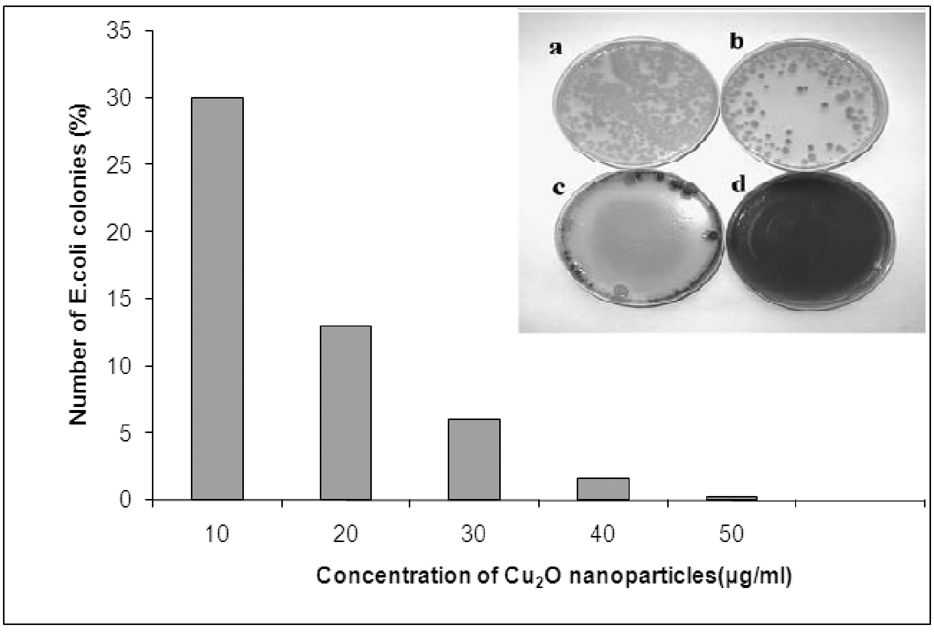

Fig 5. Number of E.Coli colonies as a function of concentration of Cu_2O nanoparticles in LB agar plates expressed as percentage of the number of colonies grown on Cu_2O – free control plates. The photograph inserted in the upper right corner shows LB agar plates containing different concentration of Cu_2O nanoparticles: a) 0, b) 30, c) 40, d) 50μgcm^{-3}.

4. Conclusion

The green chemistry approach used in the present work on the synthesis of Cu_2O nanoparticles is simple, cost effective and the resultant nanoparticles are highly stable andreproducible. Our results demonstrate the possibilityof synthesizing Cu_2O nanoparticles from the *Tridax procumbens.* Their antibacterial activity represents a significant advancement in the nanomaterials field, with realistic applications. PANI coated Cu_2O were successfully prepared by an in situ polymerization method. Polyaniline can be coated through the oxidation of aniline by using peroxide as an oxidant and the polyaniline layer can be formed on the surface of copper oxide gradually. The polyaniline coated layer is compact and final products have higher resistance in air. In the future, Cu_2O nanoparticles could replace some antibiotic medicines used to combat pathogenic bacteria in the gastrointestinal tract of animals.

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