

Research Article

Green Synthesis of Novel Jasmine Bud-Shaped Copper Nanoparticles

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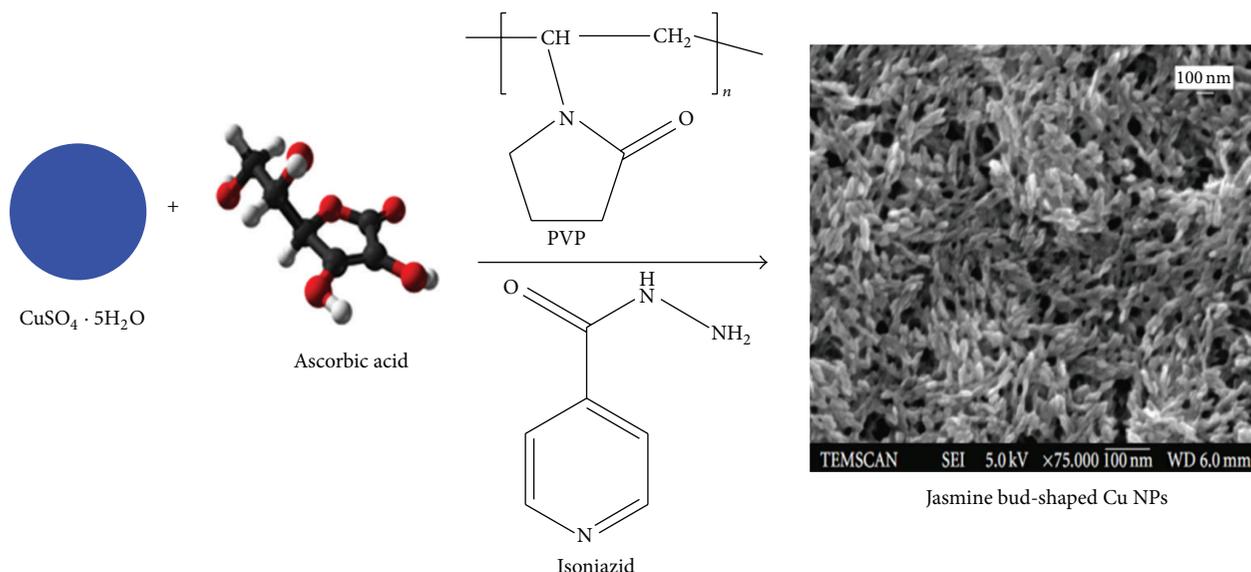
Novel jasmine bud-shaped copper nanoparticles were synthesized by a green chemical reduction method using polyvinylpyrrolidone (PVP) as a capping agent, L-ascorbic acid (AA) as a reducing agent as well as antioxidant agent, isonicotinic acid hydrazide (INH) as a reducing agent, and water as a solvent at 60–70°C (pH-7) in the presence of air. The UV-Vis absorption maximum obtained is 573 nm. The crystal lattice (fcc) structure of Cu Nps was confirmed by X-ray diffraction (XRD). The novel jasmine bud shape was visualized in a transmission electron microscope (TEM). The height of single copper nanobud was 6.41 nm as measured by atomic force microscope (AFM). The average particle size 6.95 nm is obtained by XRD results. Antibacterial activity of the Cu nanobuds was evaluated by testing against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria.

1. Introduction

Nanotechnology is one of the fast developing technologies and its products are very useful in all fields, because of their small size (10^{-9} nm) and large surface area. Nanoparticles offer a larger surface-to-volume ratio and a higher concentration of partially coordinated surface sites than the corresponding bulk materials. The unique properties of nanoparticles are due to a strong interplay between elastic, geometric, and electronic parameters. The result of these features is often improved by physical and chemical properties compared to that of bulk material [1]. The research on nanoparticles has gathered wide attention during the last decade because of their unusual and size-dependent optical [2], magnetic [3], electronic [4], and chemical [5, 6] properties. To fully utilize these properties, the size and shape must be well controlled.

Copper nanoparticles are known to be extremely sensitive to oxygen, and therefore there are several problems related to the stability and oxidation resistance. The synthesis of copper nanoparticles has not been as widely explored as that of many other metals due to the easily oxidizable nature of copper, which is enhanced in nanoscale structures. Yet, several

methods have been reported. Copper nanoparticles have been successfully synthesized, for example, by γ -radiolysis [7], laser irradiation [8], thermal decomposition [9, 10], thiol-induced reduction in supercritical water [11], reduction in microemulsions [12], and reverse micelles [13], vapor deposition [14], sonoelectrochemical [15], flame spray [16], and chemical reduction [17–19] methods. These methods are time-consuming and are carried out by using expensive instruments. Yanase and Komiyama [20] have examined the mechanism and kinetics of copper nanoparticles oxidation and reduction with UV-Vis spectroscopy by recycling glass-supported particles between oxygen and hydrogen atmospheres. Wu and Chen [21] have used CTAB as capping ligand for the synthesis of small (5.1 nm) Cu nanoparticles, which they have reported to be stable in aqueous solution for months. Copper nanoparticles are found to aggregate severely without proper protection and they oxidize easily in air. Recent studies [22] show that copper nanoparticles are, at room temperature, only oxidized from the surface. Although gold and silver withstand oxidation better than copper, copper is still a very attractive candidate for future conducting materials because of its abundance and cheapness. The problems of aggregation and oxidation can be



SCHEME 1: Schematic illustration of synthesis of jasmine bud shaped copper nanoparticles. Copper sulfate reacts with PVP to form a complex which is reduced by ascorbic acid and isoniazid resulting in copper nanobuds.

circumvented by the use of various protecting agents such as polymers [23, 24] and organic ligands. The usefulness of Cu as an antibacterial agent has been known for a long time. It is an effective agent with low toxicity, which is especially important in the typical antibacterial treatment.

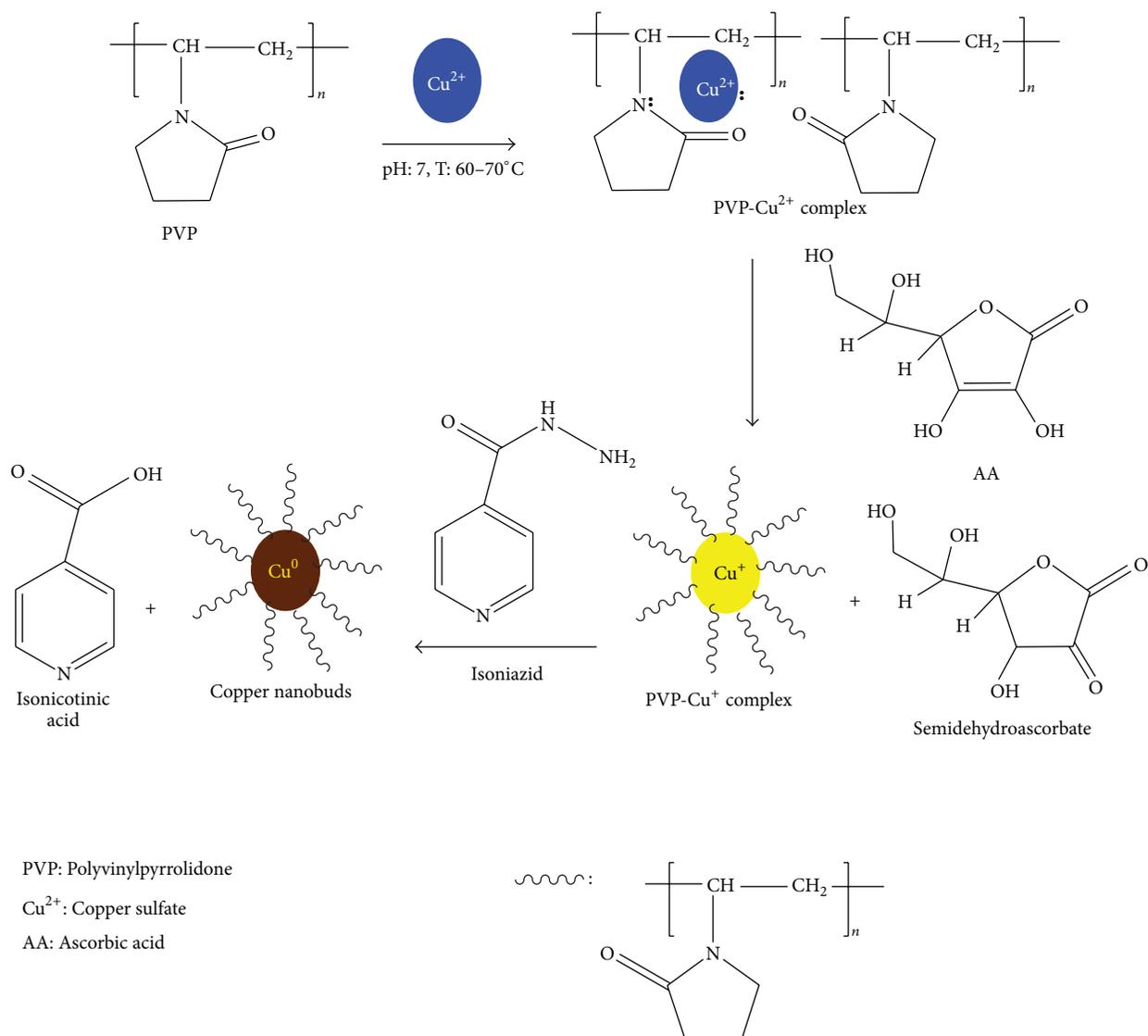
In recent years with the development of various new nanomaterials, the environmental concerns pertaining to the synthesis and environmental impacts of synthetic nanoparticles are also mounting. To ensure future sustainable development and applications of nanotechnologies, it is essential to implement the principles of green chemistry in every aspect of the nanotechnology from material manufacturing to the environmental fate of the nanomaterials [25]. Sastry et al. have reported green synthesis of Cu NPs using nontoxic and inexpensive materials like curd, milk, and herbal extracts such as tamarind and lemon juice as capping agents [26]. Typically strong reducing agents such as sodium borohydride and hydrazine are employed to prepare copper nanoparticles. These strong reducing agents create pollution associated with manufacturing of chemicals and the harmful byproducts from the particle fabrication. In the production of copper nanoparticles, the use of environmentally harmful chemicals and the associated environmental risks are minimized. In this report, the synthesis of copper nanobuds by green chemical reduction using isonicotinic acid hydrazide is described. Polyvinyl alcohol has been used as a capping agent and ascorbic acid, which is a naturally occurring material, and has been used as an antioxidant for the preparation of copper nanoparticles. The reducing nature of isoniazid, which is a biocompatible anti-TB drug, was considered to be advantageous over other reducing agents to avoid slow reaction kinetics that eliminates the formation of copper oxide nanoparticles. To the best of our knowledge, this is the first report on the green synthesis of copper nanobuds

using isonicotinic acid hydrazide. The copper nanoparticles have good antibacterial activity and antimicrobial formulations comprising nanoparticles could be used as an effective bactericidal agent [27–32].

2. Materials and Methods

2.1. Chemicals. Copper (II) sulfate pentahydrate (Merck, purity: 99%), isonicotinic acid hydrazide (Fluka, purity: 99%), L-ascorbic acid (S.D.Fine Chemicals, purity: 99%) and sodium hydroxide (Thomas Baker, purity: 97.5%), polyvinylpyrrolidone (Aldrich, purity: 98–99%) were used without further purification. All the reactions were carried out by using double distilled water.

2.2. Synthesis of Jasmine Bud-Shaped Copper Nanoparticles. The copper nanoparticles were synthesized by the following method (Scheme 1). In a typical procedure, the aqueous solution of copper (II) sulfate pentahydrate (0.01 M) was prepared by dissolving this substance in Milli-Q water (10 mL), and then it was added to a solution of 1% PVP (10 mL). Then, the solution of NaOH (0.5 M) in deionized water was added dropwise to adjust the pH to 7. The reaction mixture was continuously stirred for 1 h. Ascorbic acid (60 mg) was dissolved in 10 mL deionized water. After stirring at room temperature for 1 h in presence of air, the mixture was kept at 60–70°C for about 10 min. The ascorbic acid was added dropwise to this reaction mixture. The color of the solution turns yellow indicating the formation of copper nanoseeds. Isoniazid (0.001 M) was gradually added to this solution. The color of the reaction mixture changes to reddish brown suggesting the formation of copper nanobuds. Heating and stirring were maintained for further one hour in order to promote the growth of nanobuds. The solution was centrifuged



SCHEME 2: Mechanism of the formation of copper nanobuds. Copper sulfate forms a complex with PVP when the solution is maintained at pH-7 (60–70°C). The solution turns yellow after the addition of ascorbic acid indicating the formation of Cu(I). INH was added dropwise to this reaction mixture resulting in jasmine bud-shaped copper nanoparticles.

at 8,000 rpm for 30 min. The product was washed twice with ethanol and dried under vacuum.

2.3. Antibacterial Studies. *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were used for the antibacterial study by well diffusion method. Nutrient agar plates were prepared and incubated overnight to check for contamination and the bacterial suspension was grown overnight in nutrient broth. 4 mm diameter wells were made on the contamination free agar plates with the help of a gel punch. Bacterial suspension was swabbed on the surface of the plates with sterile cotton swabs. Control experiments were also carried out in the presence of ampicillin. 50 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$ copper nanoparticles were prepared and 10 μL of copper nanoparticles inoculated aseptically to the individual wells.

2.4. Characterization. The copper nanoparticles were characterized by following studies. The UV-Vis spectra were recorded on a Shimadzu UV-1800 UV-Vis spectrophotometer. Powder X-ray diffraction (XRD) analysis was carried out on a Shimadzu XD-D1 XRD unit with a nickel-filtered $\text{Cu-K}\alpha$ radiation at a scanning speed of $1^\circ/\text{min}$. Transmission electron microscopy (TEM) images were obtained using the JEOL 100 CXII microscope. Samples for transmission electron microscopy (TEM) were prepared by keeping a drop of the colloidal solution on a copper grid. Samples were dried and kept under vacuum in desiccator before placing them in a specimen holder. The atomic force microscope (AFM) images were recorded under ambient condition using tapping mode of a multimode imaging unit, SPA-400 (Seiko Instruments Inc., Japan) equipped with a controller SPA-4000. The AFM was used to visualize the surface morphology of the samples.

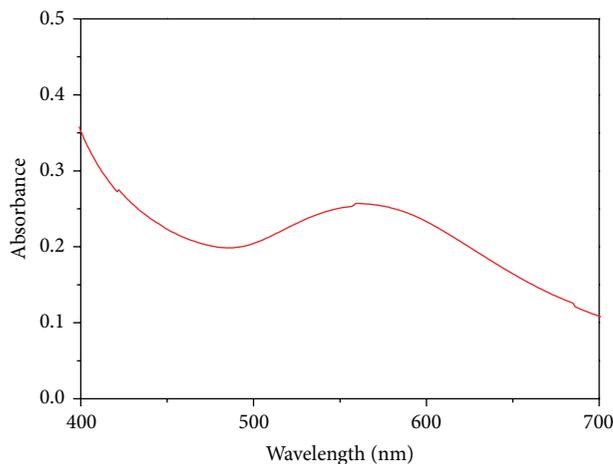


FIGURE 1: UV-Vis spectrum of copper nanobuds dispersed in deionized water at room temperature exhibiting a peak at 573 nm.

3. Results and Discussion

In the present work, Cu nanoparticles were synthesized by the reduction of Cu^{2+} using INH (Scheme 2). PVP is also known to be an excellent capping agent to prevent agglomeration and precipitation of the particles; it has been frequently employed as stabilizers in chemical synthesis of metal nanoparticles. The structure of PVP has a polyvinyl skeleton with nitrogen and oxygen polar groups, and the polar groups of PVP can occupy the hybrid orbitals of the copper ion to form a Cu^{2+} -PVP complex. The temperature of the solution was maintained at 60–70°C. The effect of temperature on the synthesis of hydrophilic CuNPs was reported by khanna et al. [33].

When the solution pH was maintained at 7.0, the formation of $\text{Cu}(\text{OH})_2$ was observed in the solution, similar to the observation made by Liu et al. [34]. Ascorbic acid is a mild reducing agent as well as an antioxidant. Ascorbic acid is essential to avoid the oxidation of copper nanoparticles during the synthesis and in storage. The antioxidant property of ascorbic acid is attributed to its ability to scavenge free radicals and reactive oxygen species [35], accompanying the donation of electron to give the semidehydroascorbate. The Cu^{2+} -PVP complex was reduced to Cu^+ -PVP complex. It forms yellow color Cu nanoseeds in the synthesis. As PVP molecule is strongly adsorbed on as-prepared copper nanobuds, they effectively prevent the aggregation in the reduced copper ions. Isoniazid is highly water soluble and a biocompatible compound. It is a mild reducing agent with a hydrazide group that helps in terminating the particle growth resulting in the formation of nanobuds (Cu^0 -PVP). The formation of copper nanobuds is indicated by the change of color from yellow to brown. INH serves as a stable donor and is converted into isonicotinic acid. It reduces the particles size below 10 nm which is stable for more than one month. AA and INH together constitute the system which is sufficient to reduce Cu^{2+} to Cu^0 . Copper nanobuds were obtained for the first time by the application of INH.

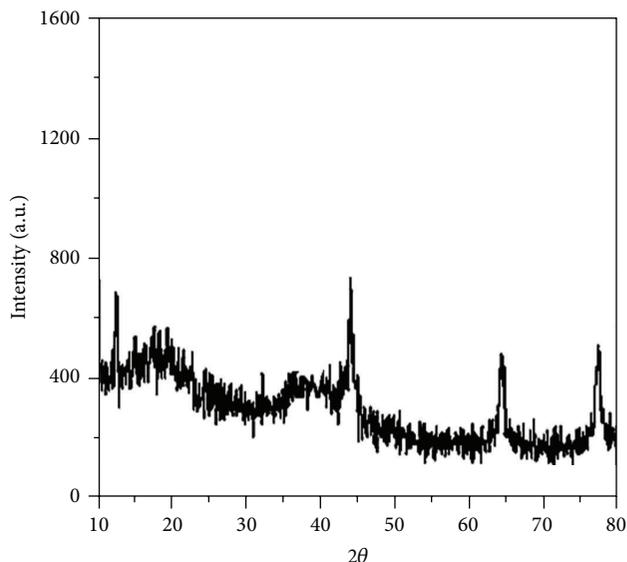


FIGURE 2: XRD pattern of PVP stabilized copper nanobuds exhibits 2θ values from 40° to 80°. The fcc structure of Cu nanobuds shows the average particle size 6.95 nm.

Figure 1 displays the UV-Vis spectrum of Cu Nps. The characteristic plasmon absorption band for copper nanobuds was observed at 573 nm. The surface plasmon resonance band for copper nanoparticles was located around 560–570 nm and it has been reported to undergo blueshift with decrease in size [36]. When the particles are spherical, the surface plasmon resonance and the blueshift are affected by the size distribution. Hence, it is suggested that the band observed at 573 nm is due to the copper nanobuds.

Stable Cu nanobuds, with sizes in the range of 10 nm, have been successfully synthesized using the green chemical reduction method. Cu Nps have been synthesized using different precursors and the spectral and microscopic data have been reported [37]. In the present investigation, Cu nanobuds have been prepared by a green chemical reduction method using copper sulfate pentahydrate as a precursor.

The XRD of Cu nanobuds exhibits peak at 43.6° and 77.7° corresponding to {111} and {220} planes of fcc structure of copper nanoparticles, whereas the 64.5° {220} plane indicates Cu_2O impurities. The XRD pattern is identified to be that of Cu Nps, as shown in Figure 2, with trace impurities of Cu_2O . All possible peaks of Cu Nps are assigned, in comparison with those described in the literature (JCPDS No-4-0836). Moderate temperature results in intensifying and sharpening of the diffraction peaks. This indicates the growth of Cu Nps grains and the nanobuds quality is improved. When the temperature is maintained at 60–70°C, the Cu nanobuds are formed. During the process of chemical reduction, the precursor decomposes to generate a nucleus of the crystal at first, and then the crystal grain grows during the thermal treatment. Thus, we can say that varying the treatment temperature can control the size of Cu nanobuds. We also use other starting materials (which are not reported here) to synthesize Cu nanobuds by the chemical reduction method.

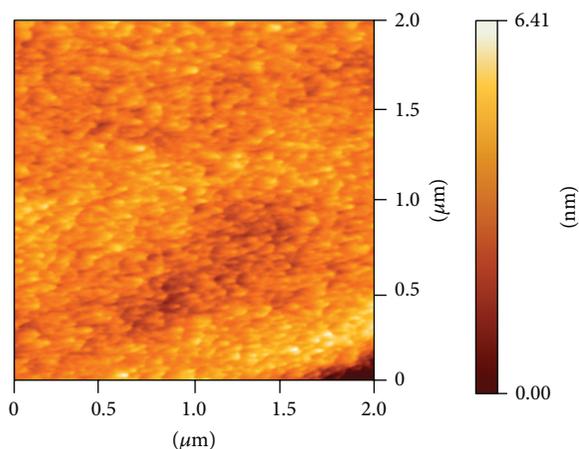


FIGURE 3: AFM 2D images of PVP capped copper nanobuds. The height of the single nanobud is 6.41 nm as measured in noncontact mode.

In fact, using precursor copper sulfate pentahydrate can result in the smallest and highest quality Cu nanobuds.

Debye-Scherrer equation is

$$D = \frac{0.9\lambda}{\beta \cos \theta}, \quad (1)$$

where D is the average size of Cu Nps, λ is wavelength (1.5409 Å), β is full width at half maximum, and θ is diffraction angle.

The size of crystallite estimated from Debye-Scherrer equation is about 6.95 nm, which may indicate a high surface area and surface area to volume ratio of the nanobuds.

Atomic force microscopy (AFM) is the technique that allows measuring interactions between various surfaces and a sharp stylus, tip. The AFM is becoming an important biophysical technique for studying the morphology of nanoparticles and biomolecules. The height and the structure of the copper nanoparticles were investigated with AFM and it indicates that the nanoparticles are bud-like in shape. The results noted in TEM images are quite agreeable to AFM observations. The shape of the copper Nps is similar to jasmine buds and the height of this particular sample is 6.41 nm (Figure 3).

TEM image for Cu nanobuds synthesized from copper sulfate is illustrated in Figure 4. It shows that copper nanoparticles exhibit bud-shape. The average particle size is approximately 7 nm, which is in good agreement with the XRD results. The XRD technique appears to slightly underestimate the particle size in the present case. The TEM image in Figure 4 indicates clearly that each particle has a bud structure.

Copper nanoparticles and the type of microorganism play an important role in the antibacterial activity [38]. The toxicity of copper nanoparticles depends on the combination of several factors such as temperature, concentration of NPs, pH, and concentration of bacteria [39]. The copper nanoparticles were tested against the Gram-negative bacterial strain *E. coli* and the Gram-positive strain *S. aureus* by well diffusion method to evaluate the antimicrobial activity of

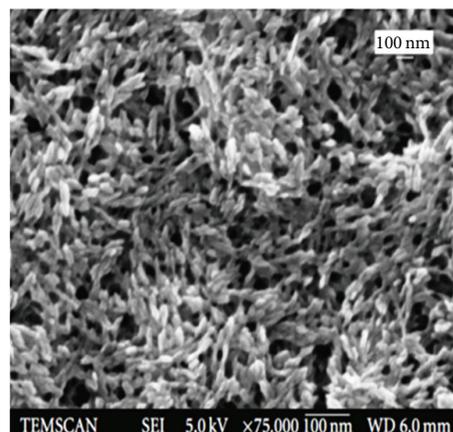


FIGURE 4: TEM images illustrate the formation of jasmine bud-shaped PVP capped copper nanoparticles in 100 nm scale.

copper nanoparticles. 4 mm diameter wells were made on the contamination free agar plates with the help of a gel punch. Bacterial suspension was swabbed on the surface of the plates with sterile cotton swabs. Control experiments were also carried out in the presence of ampicillin. Copper nanoparticles with different concentrations (50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$) were prepared and 10 μL of copper nanoparticles inoculated aseptically to the individual wells. The inoculated sets were incubated at 37°C for 24 h. The experiments were carried out in triplicates. The effectiveness of an antimicrobial agent is based on the zones of inhibition. The diameter of the zone is measured to the nearest millimeter (mm). The zone of inhibition for the *E. coli* strain for different concentrations of copper nanoparticles is 17 (50 $\mu\text{g/mL}$) and 23 mm (100 $\mu\text{g/mL}$), respectively, while that of the *S. aureus* strain are 21 (50 $\mu\text{g/mL}$) and 28 mm (100 $\mu\text{g/mL}$), respectively (Figure 5). The increase in concentration of NPs leads to higher toxicity and hence increase in the zone of inhibition. Metallic and ionic forms of copper produce hydroxyl radicals that damage essential proteins and DNA [40]. Lee et al. have reported biological synthesis of copper nanoparticles which exhibit high antibacterial activity against Gram-negative bacterium [41].

4. Conclusions

In summary, the novel jasmine bud-shaped Cu nanoparticles have been prepared by a green chemical reduction method from copper sulfate precursor. The Cu nanobuds were characterized by means of UV-Vis spectroscopy, XRD, TEM, and AFM and their antimicrobial activity has also been investigated. The results show that the Cu nanoparticles have jasmine bud-shapes and their average particle size obtained from XRD study is 6.95 nm. The presence of UV-Vis absorption peak at 573 nm is attributed to the formation of copper nanobuds which is confirmed by the TEM study. Copper nanobuds synthesized in the present investigation exhibit good antibacterial activity against the human pathogens *E. coli* and *S. aureus*.

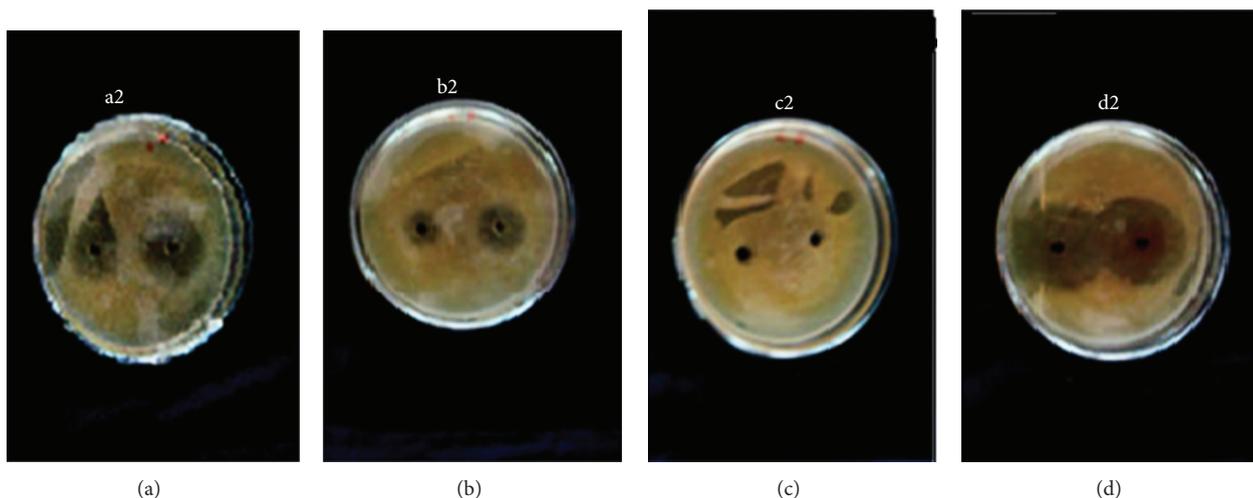


FIGURE 5: Zone of inhibition by copper nanoparticles (jasmine bud-shaped) against human pathogens—(a) *Escherichia coli*, (b) *Staphylococcus aureus*, (c) solvent, (d) ampicillin.

Conflict of Interests

The authors report no conflict of interests in this work.

Acknowledgments

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