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Synthesis and characterization of Copper Nanoparticles using Leaf Extract of *Andrographis Paniculata* and their Antimicrobial Activities.

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Abstract : Synthesis and characterization of copper nanoparticles is under exploration due to its wide medical applications and various research interests in nanotechnology. In the present investigation, an attempt was made to copper Nanoparticles is prepared by using a medicinally plant Andrographicpaniculata (Family: Acanthaceae). Copper sulphate ($CuSO_4$) was used to synthesis the copper Nanoparticles by using leaf extract of Andrographic paniculata.The structural characterization of synthesized Nanoparticles was carried out using X-RD and SEM. The optical characterization was carried out using UV – Vis analysis. The SEM results show that the copper Nanoparticles are spherical shape.The results showed that the leaf extract is optimum for the synthesis of silver nanoparticles and it is also known to have the ability to inhibit the growth of various pathogenic microorganisms. The synthesized copper Nanoparticles can be used for various applications due to its eco–friendly, non-toxic and compatibility for pharmaceutical and other applications.
Keywords: Copper Nanoparticles, Andrographic paniculata, characterization, antimicrobial activity.

1. Introduction

In recent years, Nanotechnology and Nanoparticles based product and application are increased now a days due to various fields like biotechnology, physics, chemistry, material sciences, engineering, and medicine. Copper Nanoparticles, due to their excellent physicaland chemical properties and low cost of preparation, have been of great interest.

Copper Nanoparticles havewide applications as heat transfer systems, antimicrobial materials, super strong materials, sensors and catalysts. Copper nanoparticles are very reactive because oftheir high surface, volume ratio and can easily interactwith other particles [1] and increase their antimicrobialactivities. Colloidal copper has been used as anantimicrobial agent for decades.Cu nanoparticles have attracted much attention of researchers due to its application in wound dressings and biocidal properties [2, 3], potential industrial use such as gas sensors, catalytic process, high temperature superconductors and solar cells [4,5,6]. In literature, the Cu nanoparticles are synthesized from vapor deposition [7], electrochemical reduction [8], thermal decomposition [9], chemical reduction of copper metal salt [10] and room temperaturesynthesis using hydrazine hydrate and starch [11]. In recent, green synthesis of Cu nanoparticles was achieved by using microorganisms [12], plant extract [13].Copper nanoparticles show potential antimicrobial effects against infectious organisms such as

Escherichia coli, Bacillus subtilis, Vibriocholerae, Pseudomonas aeruginosa, Syphilis typhus, and Staphylococcus aureus [14, 15]. Moreover, these nanoparticles have drawn the attention of researchers because of their extensive applications in areas such as mechanics, optics, biomedical sciences, electronics, drug-gene delivery, catalysis [16,17], photo-electrochemical applications [18], and nonlinear optical devices [19,20].

In the present work was carried out to synthesize and characterize the copper Nanoparticles using Andrographis paniculata leaf extracts.

Further copper nanoparticles were optical characterization using UV-VIS spectrometer, structural characterization using scanning electron microscopy (SEM), X – Ray Diffraction (X-RD) and antimicrobial activities.

2. Materials and Methods

2.1 Materials:

The following analytical grade materials were used without further purification: copper sulphate ($CuSO_4$). A.C.S. reagent (Sigma – Aldrich, 99% purity by wt).

Plant Materials:

The plants Andrographis paniculata leaf were collected from the cuddalore area, Tamilnadu, during January 2016.

Image of Andrographis Paniculata plant

2.2 Methods:

Preparation of the leaf extract:

Andrographis paniculata leaf were collected and used to prepare the aqueous extract.Leaf weighing 25gm were thoroughly washed in distilled water, dried, cut into fine pieces and were crushed into 100ml distilled water was added and boiled to 60ºC – 70ºC for about 10mins.Then the resulting crude extracts filtered through Whatman No.1filter paper (pore size 25µm). The filter was further filtered through 0.6µm sized filters.

Synthesis of Copper Nanoparticles:

One millmole aqueous solution of copper sulphate ($CuSO_4$) was prepared and aqueous extract of leaf of Andrographis paniculata used for the synthesis of copper Nanoparticles. 10ml of Andrographis paniculata leaf extract was added into 90ml of aqueous solution of 1mM copper sulphate. Itis kept in magnetic stirrer for 2hours at room temperature.

2.3 Characterization of Copper Nanoparticles:

2.3.1UV – VIS spectra analysis:

Synthesis of copper Nanoparticles by reducing the copper ions solutions with andrographis paniculata

leaves extract to optical characterized by using UV – Visible spectrometer, the absorption spectra was recorded using Perkin Elmer LS 45 spectrophotometer. Copper sample is dispersed in UV – Vis methanol with the help of the sonicator. The spectrum was recorded under room temperature.

2.3.2 SEM analysis of Copper Nanoparticles:

The surface morphology of the copper Nanoparticles was characterized using SEM analysis and X – RD analysis. The Scanning Electron Microscopy (SEM) used for this purpose is a Jeol – JSM – 3.5 CF – Japan.

2.3.3 X – RD analysis of Copper Nanoparticles:

The particles size and nature of the copper nanoparticles were determined using X – RD.The powdered X – ray diffraction was performed using Scifert X – ray diffractometer with a CuKα radiation.

2.4 Antimicrobial Test

The antimicrobial activity of the synthesized copper Nanoparticles is investigated against different types of pathogenic bacteria such as Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa that were cultured on agar plates added with different concentration of copper Nanoparticles by disc diffusion method.

3. Results and Discussion

3.1 Structural Characterization and Morphology:

The structural characterization was carried out using powder X – RD analysis. Fig: 1 shows typical X – RD pattern of the as-obtained copper Nanoparticles using an Andrographis paniculata leaf extract. All the diffraction peaks can be well indexed to the characteristics of cubic centred of copper Nanoparticles.

The dry powders of the copper Nanoparticles were used for X-RD analysis. The diffracted intensities were recorded from 20º to 80º at 2theta angles. The diffraction pattern is corresponds to pure copper metal powder. The X-RD pattern indicates that the copper Nanoparticles had a spherical structure. The obtained results illustrate that copper ions had indeed been reduced to CuO by Andrographis paniculata plant extract under reaction conditions. To determine the average particles size of the copper Nanoparticles, the Debye Sherrer's equation is used.

$D=K\lambda / B \cos\Theta$

Where, D is the crystalline size of Nanoparticles, K is the sherrer's constant, λ is the wavelength of the X – Ray sources used in X – RD, B is the full width at half maximum of the diffraction peak, Θ is the Bragg's angle.

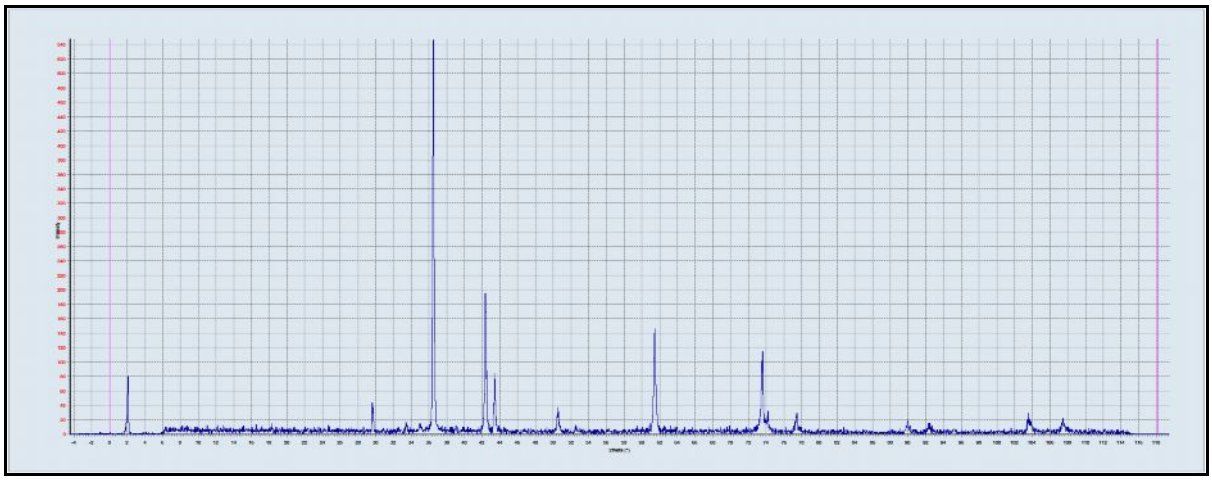
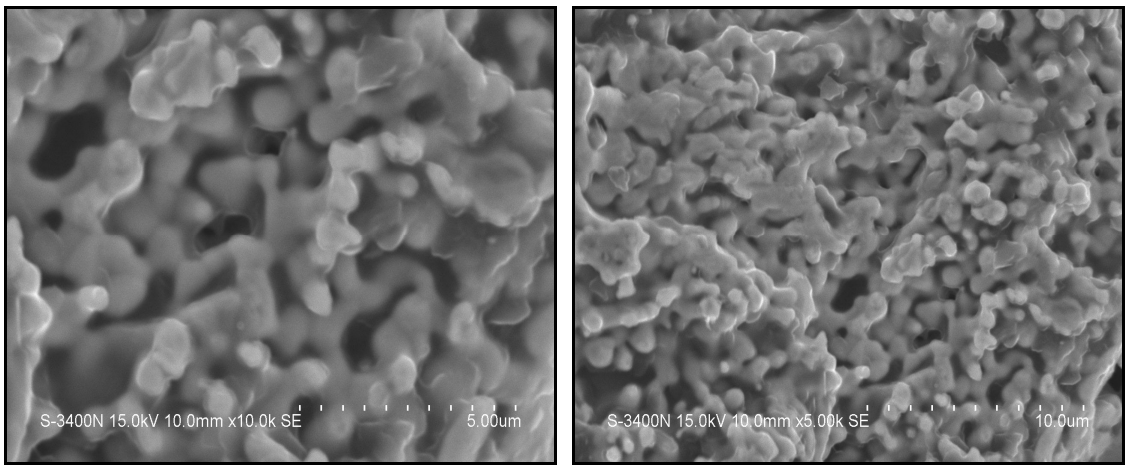


Fig: 1X – RD spectrum for the Copper Nanoparticles.

The surface morphology and size of the copper Nanoparticles was examined using scanning electron microscopy (SEM). Fig: 2 shows the scanning electron microscopy of copper Nanoparticles synthesized by the plant extract of andrographis paniculata is obtained from the proposed by bio-reduction method. The shape of the copper Nanoparticles was confirmed in the range of 5µm and 10µm.



A B

Fig: 2 A&B – SEM micrograph of the Copper Nanoparticles.

3.2 Optical characterization of copper Nanoparticles:

The brown colour crystalline of copper nano powder was insoluble in water and almost in all organic solvents. Hence a UV – Visible spectrum was recorded for the copper nano dispersed in methanol solution and it is represented in Fig: 3. it is the most important method of analysis to detect the surface Plasmon resonance property of copper Nanoparticles. UV – Visible absorption results confirmed the formation of copper Nanoparticles prepared in liquid by bio reduction method. The absorption peak observed at 232nm is the characteristics peak of copper Nanoparticles.

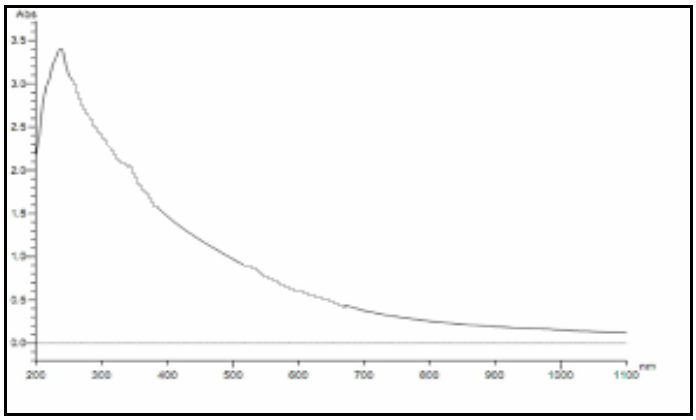


Fig: 3UV – Vis spectrum of the Copper Nanoparticles.

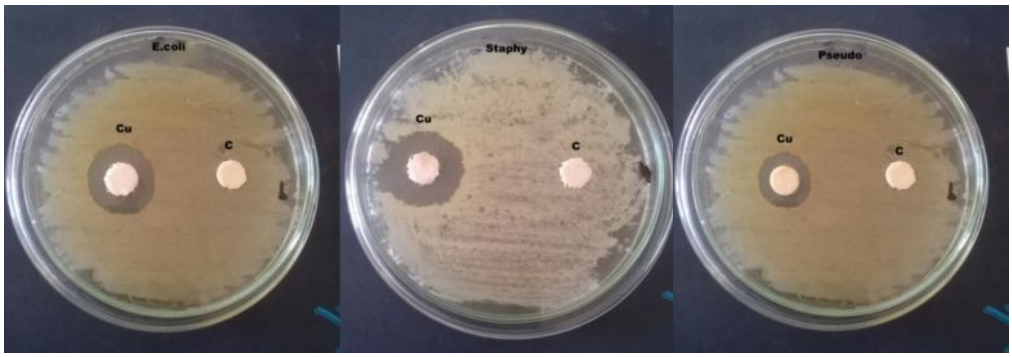
3.3 Antimicrobial Activity of copper Nanoparticles:

Copper Nanoparticles are synthesized by using andrographis paniculata plant leaves extract for the antimicrobial activity. It has been found highly toxic against pathogenic bacteria.

In this place, the copper Nanoparticles are displayed in antimicrobial activity. Fig: 5 shows copper Nanoparticles is exhibited in antimicrobial activity against both types of pathogenic bacteria such as Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa as it shown clear inhibition zone.

After the incubation period, the growth inhibition zone was measured and the results of the inhibition were measured in millimetre.

The maximum ZOI values is observed as 8mm in Escherichia coli, 10mm in Staphylococcus aureus, and 3mm in Pseudomonas aeruginosa bacteria for 50µl concentration of copper Nanoparticles as shown in the Table: 1 the zone of inhibition (ZOI) for different pathogenic bacteria of copper Nanoparticles.



A B C

Fig. 5 Antimicrobial activity of Silver nanoparticles with Escherichia coli (a), Staphylococcus aureus, (b) and Pseudomonas aeruginosa(c).

Table1: Zone of inhibition of CuNPs (mm)

S.No	Pathogenic Bacteria	50µl
1.	Escherichia coli	8±1mm
2.	Staphylococcus aureus	10±1mm
3.	Pseudomonas aeruginosa	3±1mm

4. Conclusion

In conclusion, the field of nanotechnology is the development of reliable and eco-friendly processes for synthesis of metal Nanoparticles. Here we have reported a simple biological and low cost approach for preparation of stable copper Nanoparticles by reduction of copper sulphate solution with a bio reduction method using an Andrographis paniculata aqueous extract of the whole plant as the reducing agent for their efficient antimicrobial properties.The structural characteristics and morphology of the obtained copper Nanoparticles were studied using the X – RD and SEM techniques. The result is confirmed the reduction of copper sulphate to copper Nanoparticles with high stability and without any impurity. The optical characteristics of copper Nanoparticles were studied using the UV – Vis analysis. The peak in the absorption spectrum is confirmed the formation of copper Nanoparticles. Further antimicrobial activity of plant extract and synthesized copper nanoparticles were investigated in the disc diffusion method. From the results it is clear to know that the copper nanoparticles from Andrographis paniculata plant extract also have the ability to inhibit the growth of various pathogenic microorganisms like Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa.

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