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Synthesis and Characteization of Copper Nanoparticles Using Some Plant Leaf Extracts and its Anti- Bacterial Activity

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ABSTRACT
This study the biological synthesis of copper nanoparticles using plant leaf extracts of Khat (Catha edulis), Castrol oil (Ricinus communis) and Derjihara (Prosopis juliflora) as reducing and stabilizing agents. On treatment of aqueous solutions of $CuSo_4.5H_2O$ with the leaf extracts stable copper nanopartcles were formed. UV – Visible spectroscopy was used to monitor the quantitative formation of copper nanoparticles. The synthesized nanoparticles were characterized by XRD and FTIR spectroscopy. The XRD analysis of copper nanoparticles indicated that they ranged in size from 22.32 to 2.05 nm FTIR measurements suggests that materials present in the leaf extracts have ability to bind metal particles indicating that the proteins could possibly form a layer encapsulating the metal (capping of copper nanoparticles) to prevent from agglomeration and thereby stabilize the nanoparticles. Antibacterial tests of the as-synthesized nanoparticles were carried out on Gram – negative bacteria Escherichia coli and Gram-positive bacteria staphylococcus aurous by impregnating the as-synthesized copper nanoparticles using micropipette on paper discs of 6 mm in diameter and zones of inhibition were measured after 24 h of incubation. The result showed that the synthesized copper nanoparticles exhibited a strong antibacterial activity against both Escherichia coli and staphylococcus aureus.
Keywords: Antibacterial, Biosynthesis, characterization, copper nanoparticles, surface, Plasmon resonance etc.,

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INTRODUCTION

The field of technology is one of the most active areas of research in modern material science. Nanoparticles with a size approximately extending from 1 nm up to 100 nm in length in at least one dimension [4] exhibits completely new or improved properties based on specific characteristics such as size, distribution and morphology. The application of nanoscale materials and structures in an emerging area of nanoscience and nanotechnology. Nanomaterials provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine and water – treatment [10]). Nanometerials particularly metallic nanomaterials have assumed a great deal of importance as they often display unique and considerably modified physical, chemical and biological properties as compared to their counterparts of the macroscale [1]

Copper nanoparticles

Copper (Cu) is a transition metal with a distinct red-orange colour and metallic luster having atomic number 2 and atomic mass 63.546. It is relatively more abundant metallic element of the Earth's crust having special properties of high electrical conductivity, high thermal conductivity, high corrosion resistance, good ductility and malleability and its reasonable tensile strength makes it an essential element to the functioning of society and has played several important roles in society for thousands of years. Copper is a good conductor, can joined to itself very easily and has better corrosion resistance and is a more abundant hence cheaper material to use. This properties has made copper the number one material used in modern household water piping and associated plumbing and the metal of choice for most vehicle radiators and air conditioners.

Application of copper nanoparticles: Copper nanoparticles, due to their unque physical and chemical properties and low cost preparation, have been of great interest recently.

Copper nanoparticles have great applications as heat transfer systems, antimicrobial materials, superstrong materials, sensors and catalysts. Copper nanoparticles can easily oxdize. If the application requires the copper nanoparticles to be protected from oxidation, the copper nanoparticles are usually encapsulated n organic or inorganic materials such as carbon and silica [5].

Copper nanoparticles due to their high surface to volume ratio are very reactive, can easily interact with other particles and increase their antimicrobial efficiency. Colloidal copper has been used as an antimicrobial agent for antimicrobial agent for decades. Copper monodispersed nanoparticles (2-5 nm) embedded into a polysilicate called sepiolte ($Mg_8Si_{12}O_{30}$ $(OH)_4(H_2O)_4.8H_2O$) have revealed a strong antibacterial activity and were able to decrease the microorganism concentration by 99.9% [3]. Copper inhibitory effect on the growth of microorganisms (E.Coli and S.Cerevisae) [2]. Due to the stability of copper nanoparticles supported on a matrix and their disinfection properties copper nanoparticles can be used paint or plaster as a bactericide agent to coat hospital equipment.

A major problem facing fuel-cell technologies of high levels of carbon monoxide (CO) which is produced during hydrogen production. One way to eliminate the CO by product is to combine it with water to produce hydrogen gas and carbon dioxide (CO_2) in a process known as the water – gas shift" reaction.

$$CO + H_2O \rightarrow H_2 + CO_2$$

With the assistance of proper catalysts, the water – gas shift reaction can convert a large portion of carbon monoxide into carbon dioxide. For this purpose, to achieve greatest catalytic activity, nanoparticles (2-4 nm) of either gold or copper supported on a metal oxide (zinc oxide, ZnO and cerium oxide, CeO_2) have been used. Although gold nanoparticles show the greatest catalytic activity in water – gas shift reaction, copper s almost as reactive and its cost is much lower [6].

METHODS OF SYNTHESIS

Copper nanoparticles have been synthesized via various techniques, typically categorized as physical and chemical processes. Physical methods such as laser ablation [7], vacuum vapour deposition [8] and radiation methods are capable of producing a wide range of metal nanoparticles with little effort being required to modified them for each material. The quality of produced particles is not as high as chemical synthesized ones. These physical methods usually required expensive vacuum systems to generate plasmas.

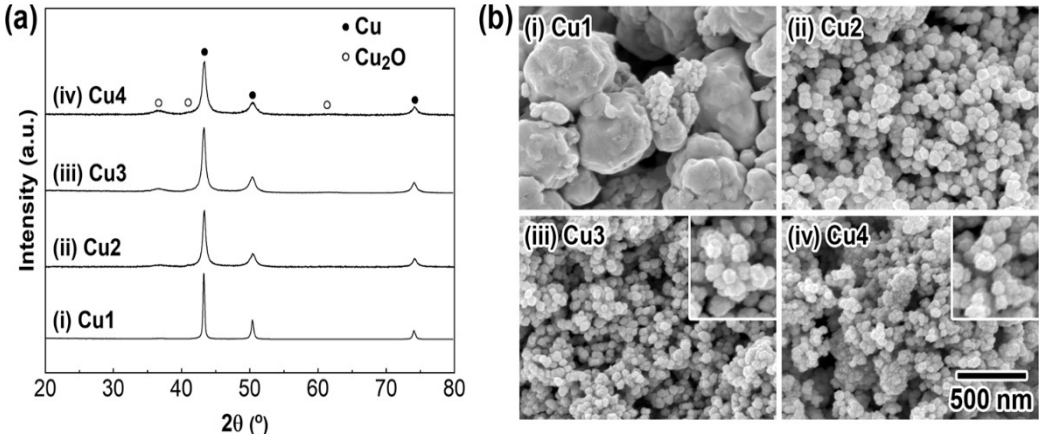

Fig.1

MATERIAL AND METHODS

Apparatus and instruments

UV – Visible spectrosphotometer (SANYO SP65 GALANAKAMP, UK): FTIR spectrometer (SHIMADZU 1730, JAPAN); XRD (BRUKER D8 Advanced XRD, West Germany); Autoclave, Incubator, Centrifuge (K3 Series, CENTERION SCIENTIFIC LTD), Pyrex glass beakers, mortao and pestle, ceramic crucibles, Volumetric flasks, Pipettes, Magnetic stirrer, Erlenmeyer flasks, water bath were used for the present study.

Chemicals and reagents

Laboratory reagent copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) as chemical precursor extra pure (98.5%), MW = 249.68 g/mol, nutrient agar media for bacteria growth; potassium bromide (KBr, 99.5%, BDH chemicals Ltd Poole, England), sterilized water, de-ionized water, plant leave extracts of Khat, Castrol oil and Dergihara (Prosopis juliflora) as reducing and stabilizing agent were used for synthesis of copper nanoparticles.

Experimental methods

Preparation of plant leaf extracts

25 gm fresh leafs of each of Khat (Catha edulis), Castrol oil (Ricinus communis) and Dergihara (Prosopis juliflora) were separately washed thoroughly with de-ionized water dirt particles if any adsorbed on the surface of the leaves and the washed samples were air – dried. The dried leaves were crushed with mortar and pestle. The mashed sample of fresh leaves was them mixed with 100 mL of sterile double distilled water (DDW) in a 250 ml Erlenmeyer flask and kept 65° C for 30 min and then filtered off using Whatmann No.1 filter paper. The resulting sample leaf extracts was stored at 40°C [9].

Biosynthesis of copper nanoparticles:

N a typical synthesis of copper nanoparticles, 10 ml of each fresh extracts was added to ml 0.01 M $CuSO_4.5H_2O$ aqueous solution and the mixture was kept at 56°C with constant stirring on a magnetic stirrer for 6 hrs. the suspension produced was centrifuged at 3000 rpm for 10 min and the supernatant liquid was decanted off and the residue was repeatedly washed with 10 ml of deionized water. Centrifugation decantation washing processes were repeatedly done six times to remove impurities if any on the surface of the copper nanoparticles. The obtained precipitate was dried n an oven at 50°C for 24 hrs. The synthesized copper nanoparticles were then kept for further characterization by FTIR, XRD and Antibacterial studies.

Methods of characterization

For XRD analysis, the dried sample of the as-synthesized copper nanoparticles was calcined at 400°C for 4 hrs n furnace. The XRD pattern of the synthesized nanomaterial was then recorded using an X – Ray diffractometer. A thin film of the sample was made by dipping a glass plate for XRD studies. The diffraction pattern was recorded with Cu targeted K radiation at a wavelength of 1.5405 Å. The scanning was done over 2 value range of 4° to 80° C at 0.02 min^1 and at 1 second time constant. The instrument was operated at a current of 30 mA and voltage of 40 kV . the crystalline domain size was calculated using Scherrer's formula.

$$D = K\lambda/\beta COS\theta$$

Where D = Average crystalline size,

λ = X – ray wavelength

β = Full width at half maximum (FWHM) of XRD spectral peak (in radians)

θ = Bragg's angle.

Fourier transform infrared (FTIR) spectroscopic studies

For FTIR measurements, the precipitate of copper nanoparticles obtained using each plant leaf extract of Khat (Catha edulis), Castrol ol (Ricinus) and Dergihara (Prosopis juliflora) were dried in an oven at 50°C for 24 hrs. the dried synthesized copper nanoparticles were then ground with KBr and casted into pellet and made ready for analysis on FTIR spectrometer in the diffuse reflectance mode operating at a resolution of 4 cm^{-1} [5].

Antibacterial Activity Studies

The antibacterial assays were done for Gran – negative Escheichia coli and Gram – positive bacteria Staphylococcus aureus by paper disc diffusion method. Nutrient agar media were used to cultivate bacteria.

Preparation of inoculums

The test bacterial srains were transferred from the stock cultures as streaked on nutrient agar (NA) plates and incubated for 24 hrs. Well separated bacterial colonies were the used as inoculums. Bacteria were transferred using bacteriological loop to autoclaved nutrient agar that was cooled to about 45°C in a water bath mixed by gently swirling the flasks. The medium was then poured to sterile petri plates, allowed to solified and used for the biotest [7].

A fresh culture of inoculums of each culture was streaked on nutrient agar media in a petridish. 10 and 20 ml aliquots containing 5 mg / ml as-synthesized copper nanoparticles were impregnated using micropipette on paper discs of 6 mm in diameter.

RESULT AND DISCUSSION

Surface plasma resonance (SPR) pattern that are characteristics of metal nanoparticles, strongly dependent on nanoparticles size, presence of stabilizer molecules and the dielectric constant of the medium. Observed surface plasma resonance bands, which appeared with increase in the reaction time may indicate the formation of anisotropic molecules that later stabilized in the medium (Krishnaraj et al 2010). The results are in agreement with the previous observations.

UV – Visible Absorption spectroscopy study

UV – Visible spectra of aqueous $CuSO_4.5H_2O$ mediated with three leaf extracts as a function of reaction time are shown in Figure. Bioreduction of Cu^{2+} ions to Cu nanoparticles using leaf extracts were indicated

by the change of color from greenish to red. The progress in reduction of copper ions to Cu nanoparticles using the three leaf extracts was indicated by the enhanced intensity of surface plasma absorption peak observed within 55 nm. Time taken for attaining maximum reduction of copper ions to from Cu nanoparticles for Khat (Catha edulis), Castrol oil (Ricinus communis) and Dergihara (Prosopis juliflora) were recorede as 165, 220 and 335 min, respectively.

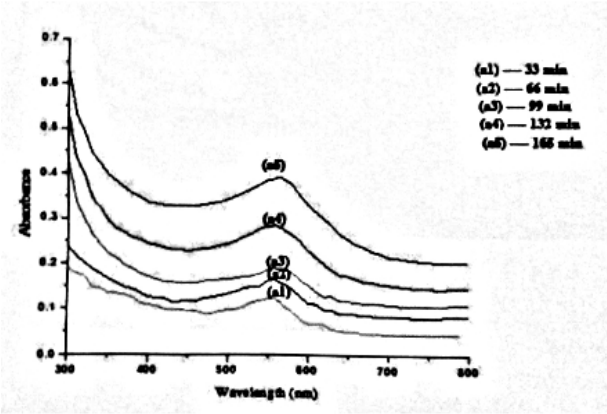

Fig. 2 UV – Visible spectra of aqueous copper sulfate pentahydrate solution mixed with Khat (Cathe edulis) as a function of time.

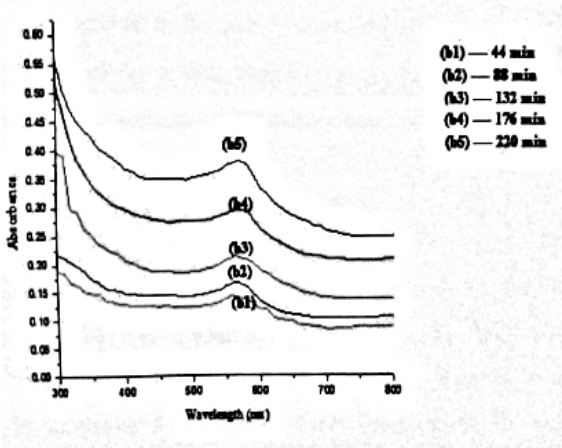

Fig. 3 UV – Visible spectra of aqueous copper sulfate pentahydrate solution mixed with Castrol oil (*Ricinus communis*) as a function of time.

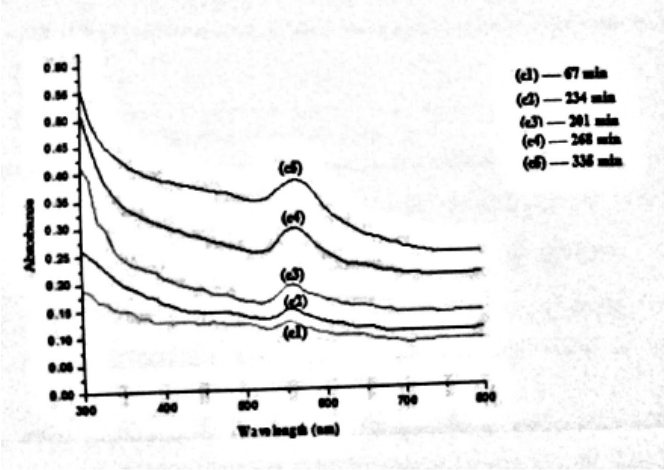

Fig. 4 UV – Visible spectra of aqueous copper sulfate pentahydrate solution mixed with Dergihara (Prosopis juliflora) as a function of time.

Strong absorption of visible radiation is shown by the metal nanoaprticle due to its induced polarization in its conduction electrons with respect to the immobile nucleus. When a particular wavelength is matched to the size of a nanoparticles, dipole oscillation is generated in the compensated from of the

induce polarization and the electrons in the nanoparticle resonate, introducing a strong absorption (Moskovits and Vlckova, 2005).

Fourier Transform Infrared Spectroscopy

IR spectroscopic measurements was carried out to elucidate the possible biomolecules present in the leaves of Khat (Cathe edulis), Castrol oil (Ricinus communis) and Dergihara (Prosopis juliflora), which may be responsible for capping and stabilizing of the Cu nanoparticles. IR spectra of copper nanoparticles observed by the reduction of copper ions using each of these three leaf extracts are presented in the table 1.

Table 1 shows FTIR absorption frequencies of the three plant leaf extract mediated copper nanoparticles. The main differences between the three spectra are the fact that peaks at 1703.42 cm^{-1} and 143.54 cm^{-1} appear only on CO and KH respectively.

Sample	Wave number (cm^{-1})
CO	3406.66, 2920.48, 1703.42, 1604.26, 1474.72, 1324.3, 1188.05, 1066.0
KH	3421.06, 2920.48, 1618.66, 143.544, 1381.96, 1074.90
DH	3406.66, 2920.48, 2848.51, 163.45, 1381.96, 1246.02, 1039.72

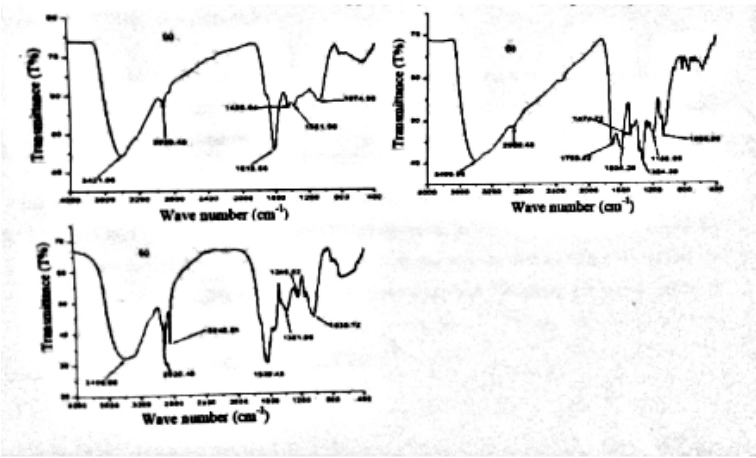

Fig .5 FTIR spectra.

The peak near 3400 and 2920 cm^{-1} are characterstics of O – H(or N – H) and aldehydrate C-H stretching. The bands at 1703.42, 163.45 cm^{-1} and 1618.66 cm^{-1} are corresponding to amide, arising due to carbonyl stretching in proteins and the bands at 1604.26 cm^{-1} is characteristics of N-H bending. The peak at 1474.72 and 103.72 and 1039.72 – 1381.96 cm^{-1} corresponds to methylene scissoring vibrations from the proteins in the solutions and C-N stretching vibrations of amine. Although not many changes were observed at the frequencies but all peaks show a shift to lower frequency and a decrease in intensity on binding with the copper nanoparticles. This suggests that free carbonyl and NH_2 groups from amino acid residues and proteins have ability to bind a metal indicating that the proteins could possibly from a layer encapsulating the metal (capping of copper nanoparticles) to prevent agglomeration and thereby stabilize the nanoparticles. FTIIR spectra show that t is the proteins molecule in the leaf extract, which possibly causes the reduction of copper ions and stabilize the Cu nanoparticles leading to their stabilizing, which are in agreement with the previous reports [1-4].

X – Ray Diffraction studies

The crystalline nature of copper nanoparticles was confirmed from the analysis of the X –Ray diffraction (XRD) patter. XRD pattern of the synthesized Cu nanoparticles obtained by bioreduction of copper ions using Khat (Catha edulis), Castrol oil (Ricinus communis) and Dergihara (Prosopis julflora) leaf extracts are presented, which were calculated using Scherer's formula are 22.32 nm, 27.96 nm and 29.06 nm. Samples XRD measurements were labeled as KH, CO and DH for Khat (Catha edulis), Castrol oil (Ricinus communis) and Dergihara (Prosopis julflora) mediated copper nanoaprticles.

Table 2. Average crystalline size of the as-synthesized copper nanoparticles using leaf extracts:

Sample	FWHM (degree)	Average crystalline size (nm)
KH	0.401	22.32
CO	0.320	27.96
DH	0.308	2.06

CO = Castrol oil (Ricinus communis), KH = Khat (Catha edulis), and DH = Dergihara (Prosopis julflora)

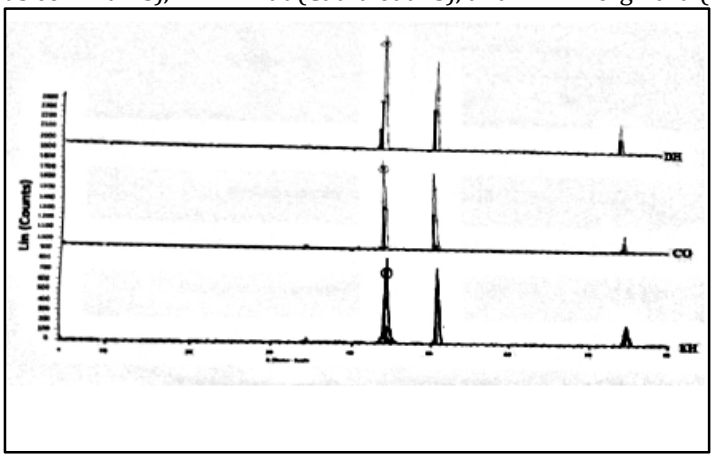

Fig.6XRD pattern of KH, CO, DH leaf extracts mediated copper nanoparticles.

The observed diffraction peaks at 2θ = 43.8°, 50.5° and 74.4° which corresponds to (111), (200) and (220) planes of copper nanoaprticles, the face centered cubic (FCC) crystal structure of as-synthesized Cu nanoparticles and results shown with previous observations (Dash and Balto, 2011; Theivasanthi and Alagar, 2011). The XRD pattern with broadening of the Bragg peaks indicates the formation of nanoaprticles.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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