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Synthesis and Characterisation of Copper Nanoparticles using *Eupatorium glandulosum* Extract and Their Antimicrobial, Antioxidant Activities.

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ABSTRACT

Green principle route of synthesizing nanoparticles have emerged as alternative to overcome the limitation of conventional methods among which plant and microorganisms are majorly exploited. Employing plants toward synthesis of nanoparticles are emerging as advantageous compared to microbes with the presence of broad variability of bio-molecules in plants. It is an important area of research in nanobiotechnology which is an emerging eco-friendly science. The present work leads to the synthesis of copper nanoparticles from cupric nitrate through the leaf extract of *Eupatorium glandulosum.* Synthesized nanoparticles are characterized under UV-Visible spectroscopy in the range of 500-600nm. Further this range and size was confirmed by Particle Size Analyzer. Organic functional groups were determined by the FTIR analysis. The synthesized copper nanoparticles showed zone of inhibition against *E.coli.* The leaf extract shows higher antioxidant activity found by DPPH and hydrogen peroxide assay. The size and the morphology was confirmed by Transmission Electron Microscopy (TEM).
Keywords: *Eupatorium glandulosum,* Particle Size Analyzer, FTIR, *E.coli,* TEM.

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INTRODUCTION

Nanotechnology as defined by size is naturally very broad, including fields of science as diverse as surface science, organic chemistry, molecular biology, semiconductor physics, microfabrication, etc [1]. In recent years, the development of efficient green chemistry methods for synthesis of metal nanoparticles has become a major focus of researchers. They have investigated in order to find an eco-friendly technique for production of well-characterized nanoparticles.

The advantages of using plant and plant-derived materials for biosynthesis of metal nanoparticles have interested researchers to investigate mechanisms of metal ions uptake and bioreduction by plants, and to understand the possible mechanism of metal nanoparticle formation in plants[2]*Eupatorium*, a genus of tribe Eupatorieae consists of about 400 species. They are mainly herbs or shrubs with opposite or alternate leaves and terminal corymbs of flowerheads, characterized by the florets, all equal and tubular, stylar arms long and obtuse, achene five angled and papus of a single circle of long scabrid hairs which differentiates it from the closely related genus *Ageratum* [3].

The seed is also carried by the wind or water and colonizes disturbed areas, such as fields and areas near to human habitation, readily. The plant is indigenous to Mexico, introduced on hill stations since 1900 and a common weed on the roadsides of Ooty in South India[4]. It is known in many other parts of the world as an introduced species and often a noxious weed.

Copper is one of the most widely used materials in the world. It has a great significance in all industries, particularly in the electrical sector due to low cost. Stability and reactivity are the two important factors that impede the use and development of the metal cluster[5].Nanoparticles of copper and its alloys have been applied more often as catalysts due to their high surface-to-volume ratio[6] and less cost compared to noble metals. They are used as water-gas shift catalysts and gas detoxification catalysts. The most critical factor of a copper-based catalyst[7] is the control of size, shape and surface properties of the copper nanoparticles.

Copper nanoparticles, due to their excellent physical and chemical properties and low cost of preparation, have been of great interest. They have wide applications as heat transfer systems[8], antimicrobial materials, super strong materials, sensorsand catalysts.Copper nanoparticles can easily oxidize to form copper oxide. To protect copper nanoparticles from oxidation, they are usually encapsulated in organic and inorganic coating such as carbon and silica[9].Copper monodispersed nanoparticles (2-5nm) have revealed a strong antibacterial activity and were able to decrease the microorganism concentration by 99.9%[10].

Antioxidants protect the human body against free radical attacks that may cause pathological conditions. Various compounds with differential antioxidant properties are found in natural resources which are considered to have high potential in the context of therapeutic approaches to prevent free radical damage[11]. The free radical scavenging potential of natural antioxidants varies among diseases and types of antioxidant[12].

MATERIALS AND METHODS

Collection of Sample

Fresh leaves of *Eupatorium glandulosum* were collected from the Western Ghats regions of Udhagamandalam, a hill station of the district Nilgiris of Tamil Nadu.Leaves were washed thoroughly and allowed for air dry in room temperature.

Preparation of Leaf Extract

The leaves were shade dried and powdered. 5g of the leaf powder was mixed with 100ml of distilled water and boiled for 10 minutes. It was filtered through Whatman No.1 filter paper (pore size 25µm) and further filtered through 0.6µm sized filters. The filtrate was used for the present study.

Chemicals

Cupric nitrate

For the synthesis of copper nanoparticles the leaf extract is supplemented with 0.1M cupric nitrate.

Synthesis of Copper Nanoparticles

30ml of leaf extract was added to the cupric nitrate. Then the sample was kept in the shaker for 48 hours. Brown colour changes will be seen after 48 hours. Thus colour change indicates reduction and copper nanoparticles were obtained.

UV-Visible Spectra Analysis

The reduction of pure copper to nanoparticle was monitored by measuring the UV-Vis spectrum the most confirmatory tool for the detection of surface Plasmon resonance property.After 48 hours, the sample was measured for its maximum absorbance using UV-Visible spectrophotometry (Hitachi U 2900).

Characterization of Copper Nanoparticles

The characterization of copper nanoparticles was done by Fourier transform infrared spectroscopy analysis (FTIR), Particle Size Analyzer (PSA), Transmission Electron Microscopy (TEM) and Atomic Force Microscope (AFM).

Fourier transform infrared spectroscopy

FT-IR is a technique which is used to obtain an infrared spectrum of absorption emission photoconductivity of a solid gas or liquid[13]. Infrared Spectroscopy (Shimadzu) gives information on the vibrational and rotational modes of motion of a molecule and hence an important technique for identification and characterization of a substance[14].

Particle size analyzer

The PSA (Nanaphox)analysis was carried out for the sample which is lyophilized and dispersed by ultrasonicator for the determination of size.

Transmission electron microscopy

The shape and size distribution of colloidal particles were characterized by transmission electron microscopy (Philips, CM-12, Netherland).An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera.

Atomic force microscope

The atomic force microscope (Nawsurf Easy Sca 2 Switzerland) is becoming an important bio-physical technique for studying the morphology of nanoparticles and biomolecules. It is a very high-resolution type of scanning probe microscopy, with demonstrated resolution on the order of fractions of a nanometer, more than 1000 times better than the optical diffraction limit. The AFM is one of the foremost tools for imaging, measuring, and manipulating matter at the nanoscale.Piezoelectric elements that facilitate tiny but accurate and precise movements on (electronic) command enable the very precise scanning. In some variations, electric potentials can also be scanned using conductingcantilevers. In more advanced versions, currents can be passed through the tip to probe the electrical conductivity or transport of the underlying surface. The tapping mode AFM images were developed especially for studying biofunctionalized samples.

Antibacterial Activity

Micro organism

The strain used for antibacterial test in the lab stocks is*E.coli.*

Culture media

Mueller hinton agar

Mueller Hinton agar was prepared with 30g of Beef extract, 1.75g of casein hydrolysate, 0.15g of starch and 1.7g of agar. The pHwas maintained at 7.3±0.2.

Antibiotic disc

The antibiotic disc used in comparison with synthesized copper nanoparticles is streptomycin.

Methodology

Bacterial sensitivity to antibiotics was determined by disk diffusion method for *E.coli.* The culture was inoculated by spread plate method. Streptomycin disc was used as standard control and distilled water was used as control for the extract. The plates were then incubated for 24 hours at 37°C.

Antioxidant Property

Antioxidant property of the leaf extract was determined by DPPH assay and Hydrogen Peroxide assay.

DPPH assay [15]

1ml of 0.1mM DPPH in ethanol was prepared. To that prepared solution plant extracts varying in concentrations from 50-250µg, 1ml ethanol and 0.95 ml TrisHcl were added. The mixture was left for 30 minutes and the absorbance was measured at 517 nm. The DPPH free radical scavenging activity was subsequently calculated.

$$\%\ \text{DPPH radical scavenging} = \frac{\text{Control OD} - \text{sample OD}}{\text{Control OD}} \times 100$$

Hydrogen peroxide assay: [15]

The leaves aliquots were taken in the different concentrations. To that 0.6ml of hydrogen peroxide with the already prepared phosphate buffer (pH 7.4) was added. The reaction mixture was incubated at room temperature for 10 minutes. After incubation Absorbance was read at 230 nm against the blank solution with phosphate buffer.

$$\%\ \text{inhibition} = \frac{\text{Control OD} - \text{sample OD}}{\text{Control OD}} \times 100$$

RESULTS

Synthesis of Copper Nanoparticles

The leaf extract were mixed with cupric nitrate at different concentrations and it is kept in the shaker. After 48 hours, there was a colour change from green to brown. This colour change indicates the formation of copper nanoparticles and thus provides a convenient means of visually determining their presence in the reaction [16].

UV-Visible Spectrometry Analysis

The samples were observed under UV-Vis spectrophotometer for its maximum absorbance and wavelength to confirm the reduction of cupric nitrate. Figure 1, study revealedthe peak range for the copper nanoparticles were found to be lied between the range of 500-600nm[17]. Hence from UV-Vis spectroscopy analysis, the wavelength for the leaf extract with synthesized copper nanoparticles was obtained between 500-600nm.

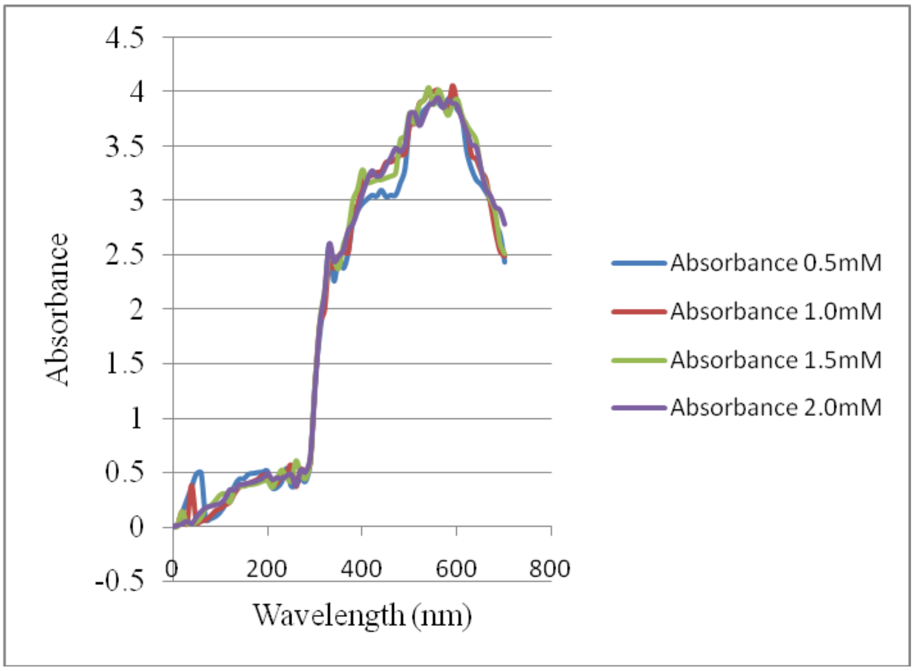

Figure 1: UV-Vis Spectroscopy absorbance for synthesized copper nanoparticles

Fourier Transform Infrared Spectroscopy

The FTIR spectra of copper nanoparticles are shown in the above figure 2. The main characteristic peaks of copper nanoparticles at 1103cm^{-1} (C-O stretch), 1381cm^{-1}(N-O stretch), 1627cm^{-1} (C=C stretch) and 3391cm^{-1} (O-H stretch, H bonded) were observed. The FTIR spectrum of a copper nanoparticle shows broad absorption bands between 2800 and 4000cm^{-1} mainly ascribed to O-H and C-O groups on the surface of the copper crystals nanostructure.

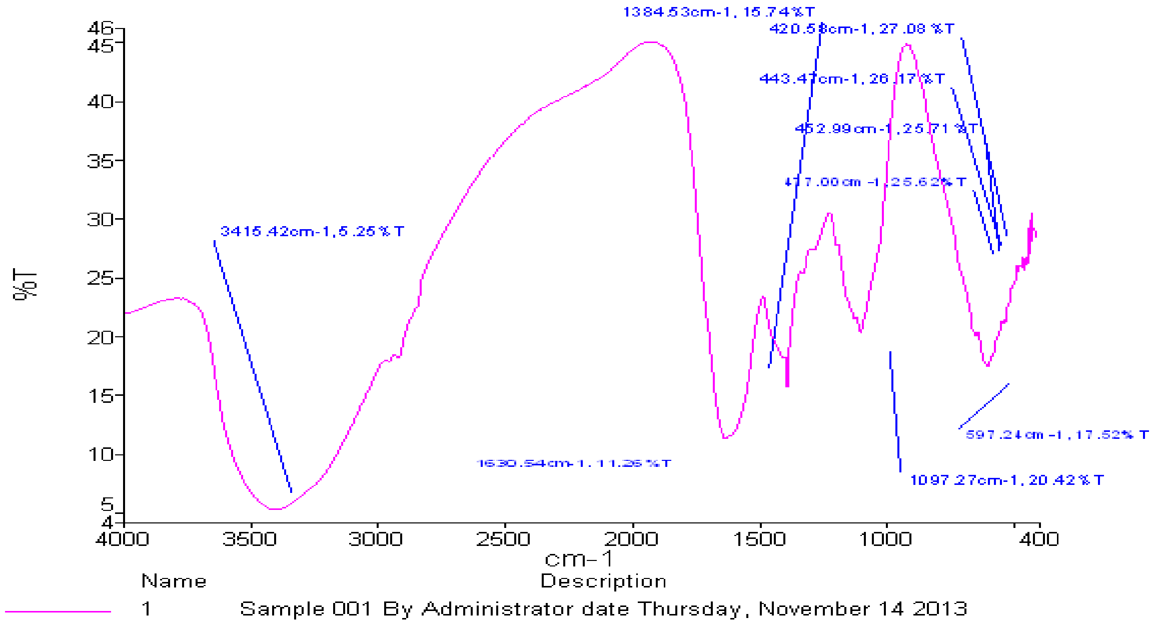

Figure 2: FTIR spectroscopic analysis of synthesized copper nanoparticles by *Eupatorium glandulosum*

The major peaks were observed to be 591cm^{-1} respectively. The peak at 591cm^{-1} should be a stretching of C-O bond [18]. The peak at this point indicated the formation of copper nanostructure.

Particle Size Analyzer

Here in the particle size analyzer result, it shows that the size range of the synthesized nanoparticle is 55.91 nm in size.Hence the synthesized copper nanoparticles were found in the figure 3 and the size range was measured within 100nm.

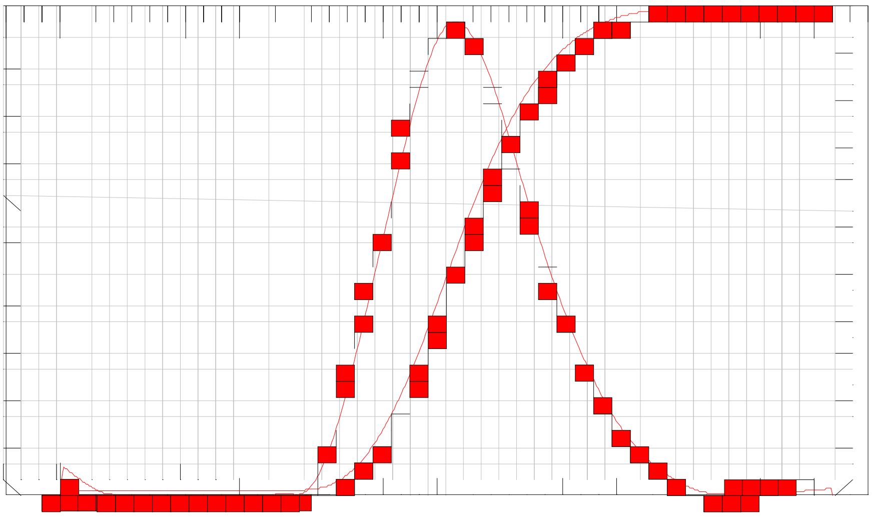

Figure 3: Particle Size Analyzer for copper nanoparticles

Transmission Electron Microscopy

Figure 4 shows the shape and size distribution of colloidal nanoparticles were characterized by Transmission Electron Microscopy (TEM) two days after preparation.

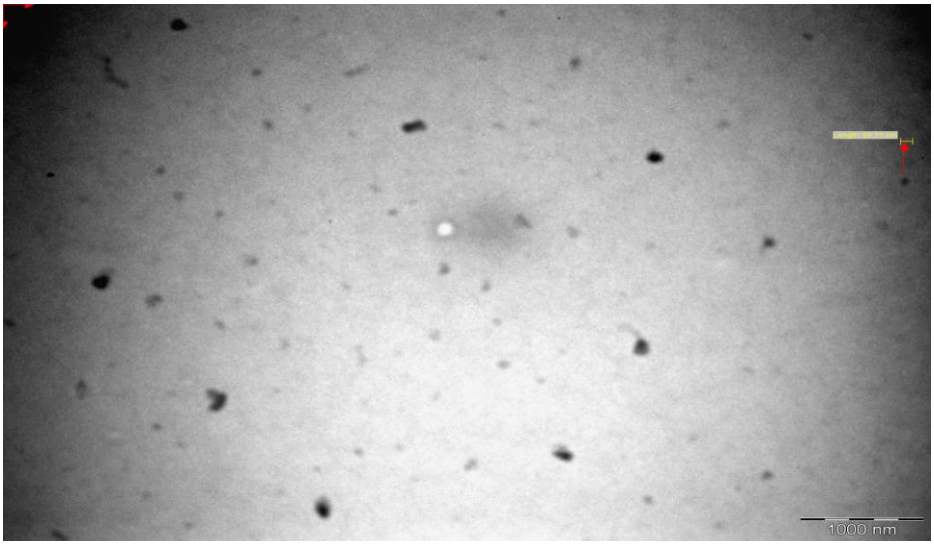

Figure 4: TEM image of Copper Nanoparticles

The TEM image of the sample showed that the average size of copper nanoparticles was about 98.70nm. From the image it is clear that the copper nanoparticles were spherical in shape and it is uniformly distributed.

Atomic Force Microscope

The AFM image was performed to visualize the two-dimensional and three dimensional topographical views of the synthesized copper nanoparticles produced by *Eupatorium glandulosum.* The image shows the synthesized nanoparticles is having the characteristics of bifunctional molecules and with the help of the topographical representation it found to be in different sizes ranges and the different sizes were seen in figure 5 and 6.

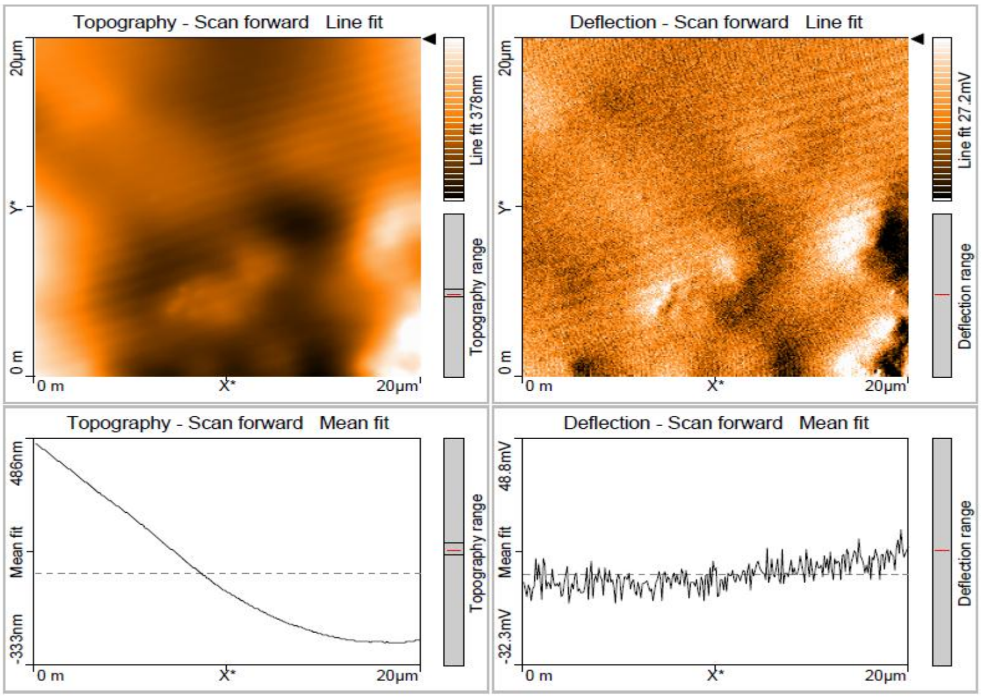

Figure 5: AFM image of copper nanoparticles

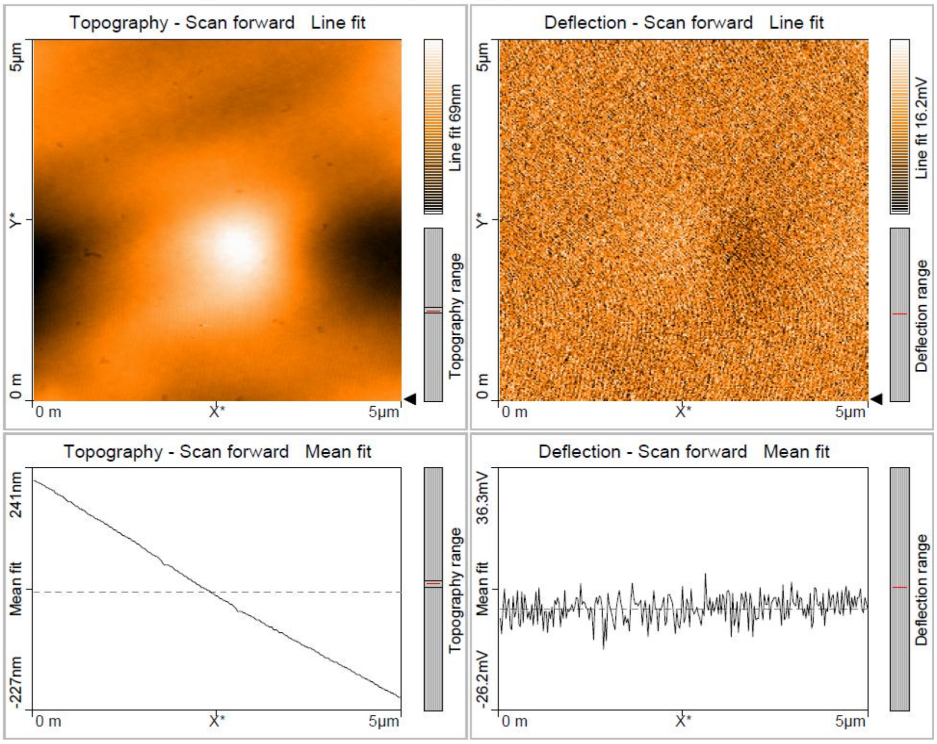

Figure 6: AFM image of copper nanoparticles

Antibacterial Activity

Figure 7 represents the antibacterial activities of the copper nanoparticles synthesized by *Eupatorium glandulosum* were compared with the commercial antibiotic disc such as streptomycin.

Figure 7: Antibacterial activity of the synthesized copper nanoparticles

The zone of inhibition was obtained. The result obtained shows best antibacterial activity of copper nanoparticles and comparing with the commercial antibiotic disc against *Escherichia coli.* This indicates the antibacterial activity of the synthesized copper nanoparticles. [19] demonstrated that highly reactive metal nanoparticles exhibit excellent biocidal action against bacteria.

Antioxidant Activity

The plant extracts were tested in different concentrations against DPPH and Hydrogen peroxide assay to find the radical scavenging activity. Free radical scavenging activity of the copper nanoparticles on DPPH radicals was found to increase with increase in the concentration according to [20]. Tables 1, 2 and the Figures 8,9 may represents the antioxidant properties of the given compound.

Table 1: Percentage of Radial Scavenging Activity

Extract	% of inhibition
0.5mM	6%
1.0mM	26%
1.5mM	52%
2.0mm	71%

Table 2: Percentage of Inhibition

Extract	% of inhibition
0.5Mm	15.53%
1.0Mm	50%
1.5Mm	50.56%
2.0mm	62.71%

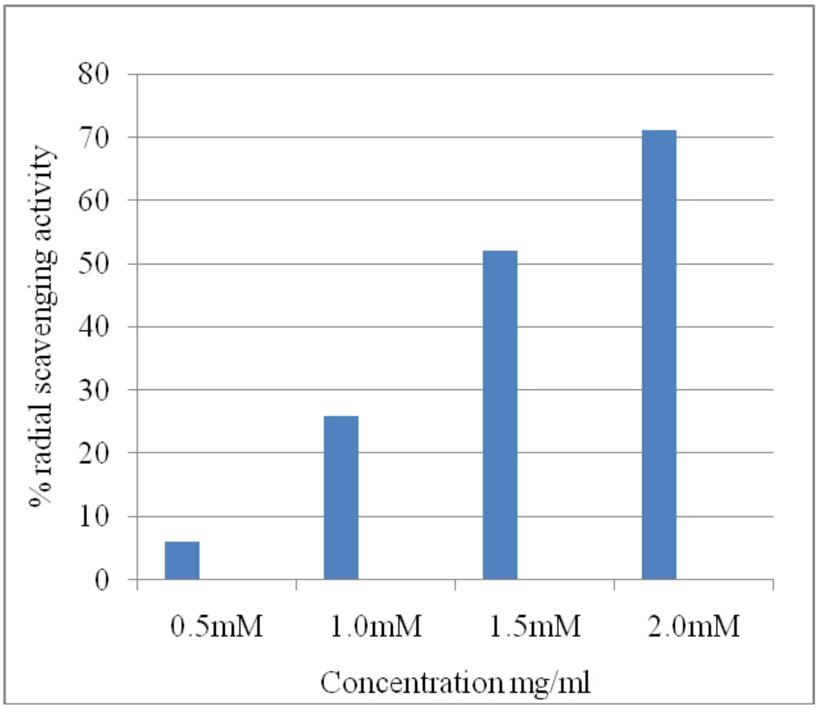

Figure 8: Scavenging activity of DPPH assay

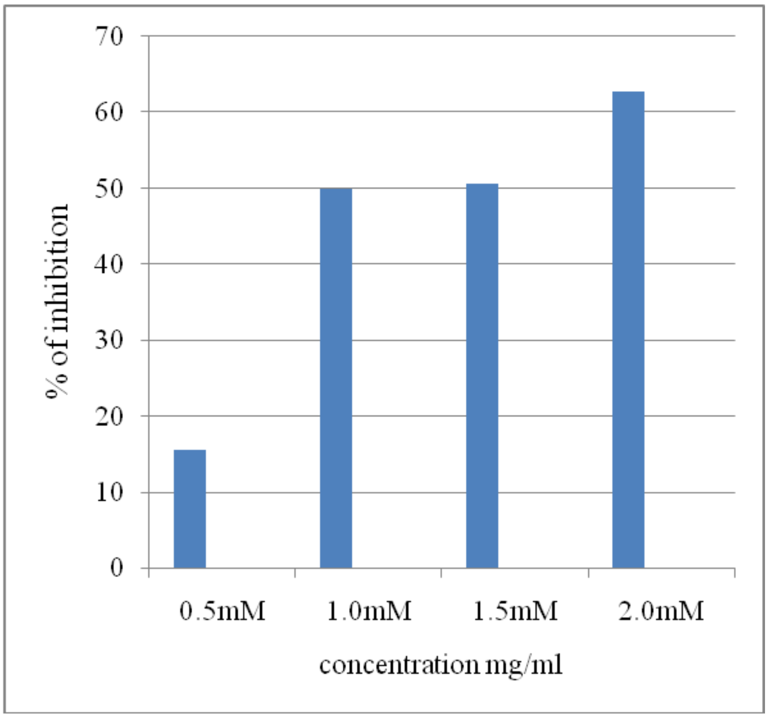

Figure 9: Hydrogen peroxide assay

DISCUSSION

Synthesised copper nanoparticles shows the strong resemblance on the determination of the functional groups by determining through FTIR, particle sizes where measured through the Particle Size Analyser. Size and shape of the particles where identified through TEM. Antibacterial and antioxidant activity against the different organisms and different compounds.

CONCLUSION

This study shows the synthesis of copper nanoparticles from *Eupatorium glandulosum.* The characterization of copper ion and the reduction of cupric nitrate to copper nanoparticles were confirmed by UV-Visible spectrophotometer. The bending vibrations and stretching bonds that are present in the sample was confirmed by the Fourier Transform Infrared spectroscopy (FTIR). The size of the particle was analyzed with the help of Particle Size Analyzer (PSA) and revealed that the particles that are present in the sample was in nanometer range. The size and shape of the nanoparticles were characterized using Transmission Electron Microscopy (TEM). The two dimensional and three dimensional visualization were studied by Atomic Force Microscope (AFM). The result shows that the plant was capable to produce copper nanoparticles. The antibacterial activity for the synthesized copper nanoparticles was confirmed by disc diffusion method. The formed nanoparticles are highly stable and have significant antibacterial action. The antibacterial activity experiment performed on *E.coli* clearly demonstrated that the smaller particle sizes of synthesized copper nanoparticles have higher antibacterial effects and higher zone of inhibition. The antioxidant property showed the highest percentage of inhibition.

Thus it is proven from this study that the copper nanoparticlessynthesized from *Eupatorium glandulosum*leaf extract seem to be promising and effective antibacterial agentagainst bacterial strains and potent antioxidant. This biological chemistry approach towards the synthesis of copper nanoparticles is highly essential effort being addressed in nanomedicine and cancer treatment because of its varied advantages.

Plant extractbeing very eco-friendly and cost effective can be used for the large scale synthesis of copper nanoparticles innanotechnology processing industries.Based on the result obtained it can be said that the plant resources could be utilized in various fields such as biomedical, nanotechnology and so on. This biosynthesis technique can be a promising method for the preparation of other metals and metal nanoparticles and can be valuable in environmental, biotechnological, pharmaceutical and medical applications.

Synthesis of metal nanoparticles using biological systems as an efficient sink has grabbed attention due to their anomalous optical, chemical, photo electrochemical and electronic properties. Unlike chemical protocols which demands expensive instruments and results in release of inimical chemicals, biological method is more facile, eco-friendly and results in more monodispersed nanoparticles.

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