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Anti-Microbial Activity and Green Synthesis of Copper Oxide Nanoparticles by Utilizing Agricultural Waste from *Musa Paradisiace* Peduncle Latex

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ABSTRACT
The research report of this paper clarified about successful synthesis of Copper oxide nanoparticles by green synthesis way and employing aqueous latex of Musa paradisiaca extract with copper sulphate. Here, synthesized nanoparticles were characterized with the help of Ultraviolet-Visible spectroscopy, Powdered X-ray diffraction (P-XRD), Fourier transform infrared spectroscopy (FTIR), X-ray Energy Dispersive Spectroscopy/Energy Dispersive Spectroscopy (EDS), Particle size analyzer and also facilitated their size and morphology by Transmission electron microscopy (HRTEM). The synthesized copper oxide nanoparticles have shown 8 nm as average particle size. In the synthesized nanoparticles from plant extract, the biomolecules are responsible to act as reducing and stabilizing agents. The CuO Nps were further analyzed for antibacterial effect with four types of bacteria. The current research of manuscript revealed that the copper oxide nanoparticles are effective growth inhibitors against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and klebseilla and also finally illustrated good antimicrobial properties.
Keywords*: Green synthesis, Musa paradisiaca peduncle latex, Copper Oxide nanoparticles, mono clinic structure, and Antimicrobial activity.*

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INTRODUCTION

In modern years, the interest in nanomaterials has been enhanced significantly owing to the small size, by comparing to bulk materials the nanomaterials exhibit unique physical and chemical properties [1]. Especially, inorganic metal nanoparticles such as silver [2], [3], copper [4],[5], platinum [6], titanium [7], zinc [8], gold [9] and iron [10] are one of the majority attractive materials due to their applications and constructive collision on pathogenic microorganism. They are used as everyday products such as makeup, personal care products, detergents, food package, cloths, and pharmaceuticals [11]. Among these, Cooper oxide (CuO) has been known as a p-type semiconductor material with narrow band gap because of the natural abundance of its starting material, low cost production processing, non-hazardous nature, and its reasonably good electrical, optical and several health related applications [12]. Due to the higher surface to volume ratio means that much more catalyst is actively participating in the reaction. The potential for cost saving is marvelous from a material, equipment, labour, and time stand point. Higher selectivity means less misuse and fewer impurities, which could lead to safer and reduced environmental impact [13]. Copper oxide nanoparticles have attracted significant attention because of their wide range of applications such as superconductors [14], sensors [15], catalytic [16], optical [17], electrical [18], gas sensors [19], solar energy conversion and preparation of organic–inorganic nanostructure composites. Further it can be used as an antimicrobial, anti-biotic and anti-fungal agent when incorporated in coatings, polymer membranes [20], plastics, textiles etc[21]. Gallo et al were successfully reported that the antibacterial coatings developed on PGLA sutures may offer important advantages in terms of prevention from surgical infections and wound curative process, and suggested a narrative approach towards antibacterial biomaterials for clinical practice [22].

Deoker et al were successfully reported that Zn-doped CuO NPs synthesized by ultrasound assisted technique with an optimized 1:3 molar ratio of precursor (Zn acetate: Cu acetate) in ethanol: water solvent. The synthesized Zn–CuO based ointments are able to prevent and disrupt the bio-film formation of three major pathogens. These results provide proof of concept that Zn–CuO NP-based ointments may be effective in treating wound infections based on alternative and non-antibiotic means [23].Peszke etal were reported the copper oxides and bimetallic silver–copper nanoparticles were synthesized by chemical reduction method. The synthesized nanoparticles show promising killing (or) inhibiting properties on Gram-negative and Gram-positive bacteria [24]. There are number of methods for the preparation of metal nanoparticles, such as photo-irradiation [25], radiolytic reduction [26], sono chemical method [27], micro emulsion technique [28], polyol process [29], hydrothermal method[30], and chemical reduction [31], above these methods have some limitations like to maintain high pressure, temperature and using toxic chemicals. To overcome these problems, recently researcher have shown great interest to develop safe, eco-friendly alternative approaches in synthesis of nanoparticles among which biological systems have been focused and exploited as a preferred green principle process for synthesis of nanoparticles. Plant contains abundant natural secondary metabolites such as alkaloids, flavonoids, saponins, steroids, tannins and other nutritional compounds. These natural phyto-chemical compounds are derived from various parts of plant such as leaves, stems, roots shoots, flowers, barks, and seeds. Recently, many studies have proved that the plant extracts act as a potential precursor for the synthesis of nanomaterials in non-hazardous ways [32]. Recently Abboud et al synthesized CuO NPs of dimensions 5–45 nm by using brown alga extract (*Bifurcaria bifurcata*) [33] , Jayakumarai et al synthesized monodisperse CuO NPs size less than 100 nm using *Albizia lebbeck* leaf extract [34], Sharma etal Synthesized CuO NPs the average particle size to be 20 nm with leaf extract of *Calotropis gigantean* [35], Sinha et al synthesized Cu NPs using the peel extracts of *Citrus grandis* the synthesized Cu NPs are 18 nm [36], Kaur et.al were successfully synthesized Cu NPs using peel extract of *Punica granatum*. The synthesized Cu NPs are ranged in size from 15 to 20 nm [37]. Rajesh et al, Cu NPs were synthesized using *Syzygium aromaticum* (clove) bud extract through simple and eco-friendly green route. The synthesized Cu NPs are almost all spherical in nature with an average diameter of ~15 nm. The bio-reduced Cu NPs exhibited an excellent bactericidal effect against *Bacillus spp.* and fungicidal activity [38]. Nasrollahzadeh et al were reported Green synthesis Cu NPs via *in situ* reduction of $CuCl_2$ in *Plantago asiatica* leaf extract. The synthesized Cu NPs were revealed spherical morphology and particle size in the range of 7-35 nm [39]. Khani et al were reported green synthesis of Cu NPs by using *Z. spina-christi* fruits extract. The size of synthesized Cu NPs was in the range of 5–20 nm and spherical in shape. The ability of Cu-NPs synthesized with *Z. spina-christi* fruits extract as a green and efficient adsorptive nano-adsorbent has been studied to remove of CV from aqueous solution and anti-microbial activity [40]. Mukhopadhyay et al were reported synthesis, characterization and evaluation of anti-melanoma potentials of biosynthesized copper nanoparticles from the floral extract of *Quisqualis indica* plant [41]. From literature survey, it was seen that naturally available agricultural wastes have not been studied for the synthesis of metal nanoparticles. A classical example of such, as plentifully available natural material is the *Musa paradisiaca* latex yielding from the cutting of peduncle after ripening of bananas. *Musa paradisiaca* popularly recognized as banana is a perennial tree-like herb cultivated in many tropical and subtropical regions around the world. Banana, eaten as a fruit or a vegetable, is one of the most important crops in several countries due to its enriched food and resourceful medicinal value. Various parts of the *Musa* plants have been used orally or topically as remedies in folk medicine and some studies have demonstrated this medicinal potential. The fruits, peel, leaves, roots and pseudo stem of *Musa* plants have shown anti ulcerogenic, antioxidant and antimicrobial activity, among others activities. In addition, studies have shown that some species of *Musa* possess anti diabetic, anti hyperglycemic and hypoglycemic activity [42], [43]. Ashok B, et.al demonstrates for the synthesis of silver nanoparticles using an extract derived from banana peels [44]. The present study reports, the synthesis and optimization of copper oxide (abbreviated CuO NPs) nanoparticles using latex of *Musa paradisiacal* peduncleand copper sulphate. The spectroscopic methods such as UV-visible, FT-IR, SEM, EDX and XRD analyses were used for characterization of synthesized CuO nanoparticles. Also, the antibacterial characteristic studies of the synthesized nanoparticles were evaluated in this study.

MATERIAL AND METHODS

Materials

Copper (II) sulphate pentahydrate ($CuSO_4.5H_2O$) was purchased from Sd. Fine Chemicals, Mumbai, India. Peduncle latex of *Musa paradisiaca* was collected early in the morning. Crude white latex was obtained by cutting the green peduncle of *Musa paradisiaca* plants and its plant latex was received from near crop fields in the Pulivendula, Kadapa District, Andhra Pradesh, and India. White slurry latex was stored at 4^0C for

further experiments. All the chemicals and reagents used in this research were of analytical grade. All aqueous solutions in the present study were prepared by using double distilled water.

Preparation of latex extract

In a typical reaction, 30 mL of crude latex was taken in to conical flask of 200 mL by 70 mL of double distilled water to make it 30% and boiled the combination at 80^0C for 30 min with constant stirring under magnetic stirrer. Through Whatman No. 1 filter paper the extract was filtered and collected. For further analysis, the refrigerated filtrate was utilized.

The 5 mM $CuSO_4.5H_2O$ aqueous solution was normalized in 100 ml of usual std. flask. A 30 ml of the *Musa paradisiaca* plant latex extract is prepared was mixed with 70 ml of $CuSO_4.5H_2O$ solution kept under magnetic stirring at 70-80^0C for 1h, finally CuO NPs formed within 1 h. The reduction of Cu^{2+} to Cu^0, awaiting the color of the reaction mixture changed from light blue to reddish. The formation of copper nanoparticles was indicated by manifestation of red color. The UV–visible spectrophotometer is used for monitoring the colloidal solution (Surface Plasmon Resonance). The content was washed with double distilled water thrice by repeated centrifugation at 8000 rpm for 15 min. these nanoparticles are again washed with methanol. In the Petri-dishes the collected copper nanoparticles were placed and dried at 70^0C for overnight, collected CuO NPs were calcinated at 400^0C for 3 h for complete removal of plant material, stored caped sample voiles properly and kept in desicator for future use.

Characterization of Synthesized CuO NPs

The formation of Cu^{2+} to Cu^0 nanoparticles was monitored by UV-Vis spectrophotometer (Lab India, Model 3092, Mumbai) range from 300 to 700 nm, by diluting 1:10 ratio of the reaction mixture in distilled water.The phase identity and crystalline nature of CuO Nps were described by X-ray diffraction technique (XRD), the XRD outline of desiccated CuO NPs recorded on Rigaku mini 600 using Cu- Kα radiation X-ray diffractometer. Dried CuO NPs were grained with KBr to make pellet and Fourier Transforms Infra-Red spectroscopy (FTIR) spectra was recorded using Perkin Elmer Spectrophotometer in the region of 4000-400 cm^{-1}. The shape, size and surface morphology of the synthesized CuO NPs were studied by using Field emission scanning electron microscopy with energy dispersive X-ray analysis (FESEM EDAX) was carried out on SUPRATM55 with co-relatively microscope SEM machine. Energy dispersive X-ray spectra (EDAX) taken along with SEM images to find out the chemical composition. The synthesized CuO NPs dispersed in distilled water and a drop of the aqueous dispersion placed on 200 mesh carbon coated copper grids and dried at ambient conditions for 10-12h. Transmission electron microscopy images were acquired using a JEOL 3010 at 200 Kv microscopy. With Zeta Sizer model Nano-S90 (Malvern U.K), the average particle size and distribution of manufactured CuO NPs was determined using nanoparticles dispersion.

Antimicrobial activity

Antimicrobial activities of the bio-synthesized CuO NPs were investigated by using disk diffusion method [45]. 10 µL of four (*Pseudomonas aeruginosa*, *Staphylococcus aureus, klebseilla*and *Escherichia coli*Sps) 24h active microbial cultures broth was spread using sterile L-shape spreader on Muller Hinton agar plates. In the 10 min. time the spread plates were permitted for standization. The disks of 5 mm size were prepared sterilized and placed on the surface of the medium in all plates using sterile forcipes. A 1 mg/mL of the CuO NPs were dispersed well in double distilled water by sonication. Three different concentrations (10, 20 and 30 µL) of CuO NPs suspension and positive control (30 µL) (Streptomycin) were prepared. With the help of micropipette in all plates, positive control and the three different concentrations of nanoparticles solutions were poured onto the disks. At 37^0C, all the plates were kept in favor of incubation for 24 h. The zone of inhibition was measured after incubation. Every screening test was conducted with three replicates.

RESULTS AND DISCUSSIONS

In this research work, *Musa paradisiaca* latex extract is used as a both reducing and stabilizing agent for production of CuO NPs. When *Musa paradisiaca* latex extract was mixed with solution of copper sulphate under continues stirring at 70-80^0C for 1h, and the reaction mixture within 10 min. starts colour change. The formation of CuO NPs was primarly confirmed by the reaction mixture color change [46]. From Figure. 1, it is indicates that the configuration of copper oxide nanoparticles by the colour change from blue to reddish and this colour change was due to the excitation of SPR of CuO NPs [34].

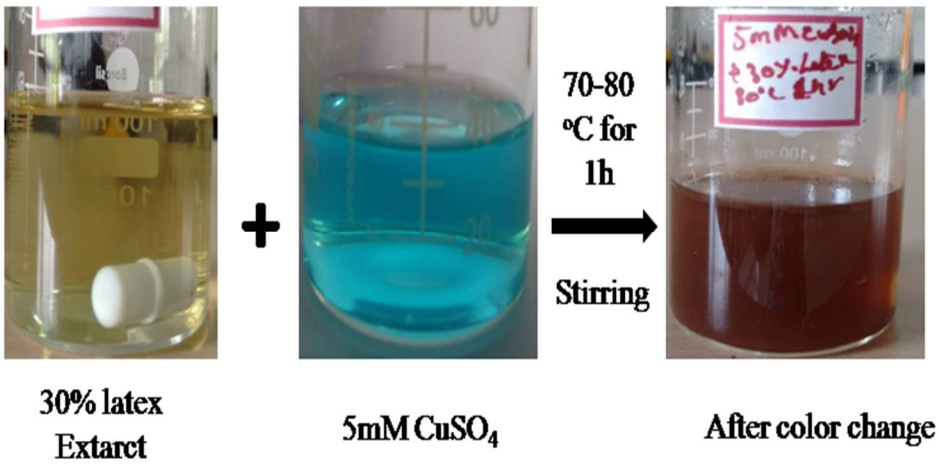

Figure.1: Shows the visual observation of color change

The UV-Vis spectrophotometer is the primary and confirmatory analytical tool for the detection of formation Cu^{2+} to Cu^{0} nanoparticles [47]. Figure. 2 depicts the UV-visible absorption spectrum of CuO NPs prepared from copper sulphate before and after calcination. Presently, UV-visible absorption spectrum is recorded between absorbance and wavelength from 400 to 700 nms. The synthesized copper nanoparticles have shown UV-Vis absorption spectra at 600 nm under room temperature and 620 nm at 90^{0}C with in 1 h, which is shown in the Figure. 2. The peaks were shown blue shift at 90^{0}C and broadened red shift at room temperature. The observed red shift implies that the particle size increases at low temperature. This spectrum confirms the presence of CuO only, as there is no other measurable peak is observed. Due to the property of Surface Plasmon Resonance (SPR), noble metal nanoparticles are known to exhibit unique optical properties can be finding in the optical absorptionspectra and which is reported in the literature [48].

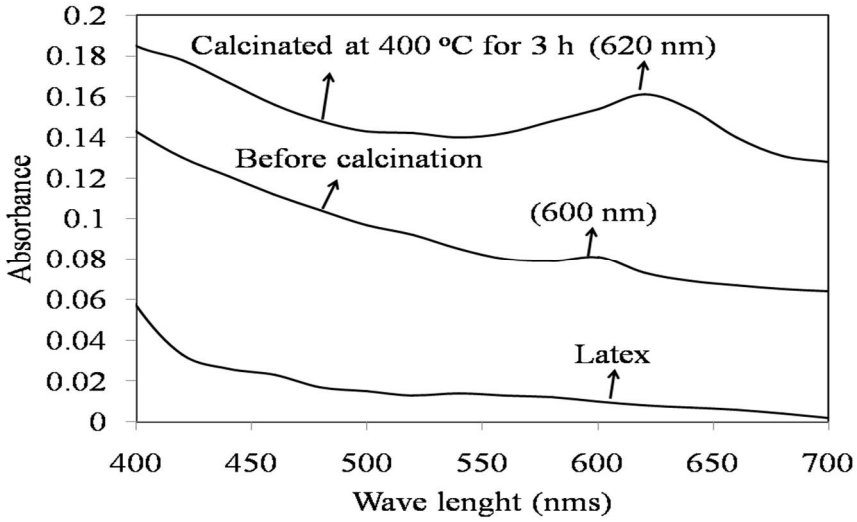

Figure. 2: Synthesized CuO Nanoparticles UV-Visabsorption spectrum

The identification of bio- molecules which are responsible for the reduction and stabilization of the synthesized CuO NPs [52] is carried out with FT-IR studies. A FTIR spectrum of *Musa paradisiaca* plant extract and green synthesized copper oxide nanoparticles in the range of 500–4000 cm^{-1} at room temperature is presented in Figure. 3.The bands were found at 3423 and 2924 cm^{-1} have been signifying to stretching vibrations of amines and primary alkanes [16] respectively. From the spectrum, metal–oxygen (M–O) is seen at 471 cm^{-1}. Strong bands were observed at 820, 1022, 1115, 1383 and 1446 cm^{-1} and have been referred to as alcohols and phenolic groups, C–N stretching vibrations of aliphatic and aromatic amines [35], respectively. The peak is obtained around 1723 cm^{-1} is due to the amide bonds of enzymes or proteins [36]. As per results, it is confirmed that the formation of CuO nanoparticles with *Musa paradisiaca* latex.

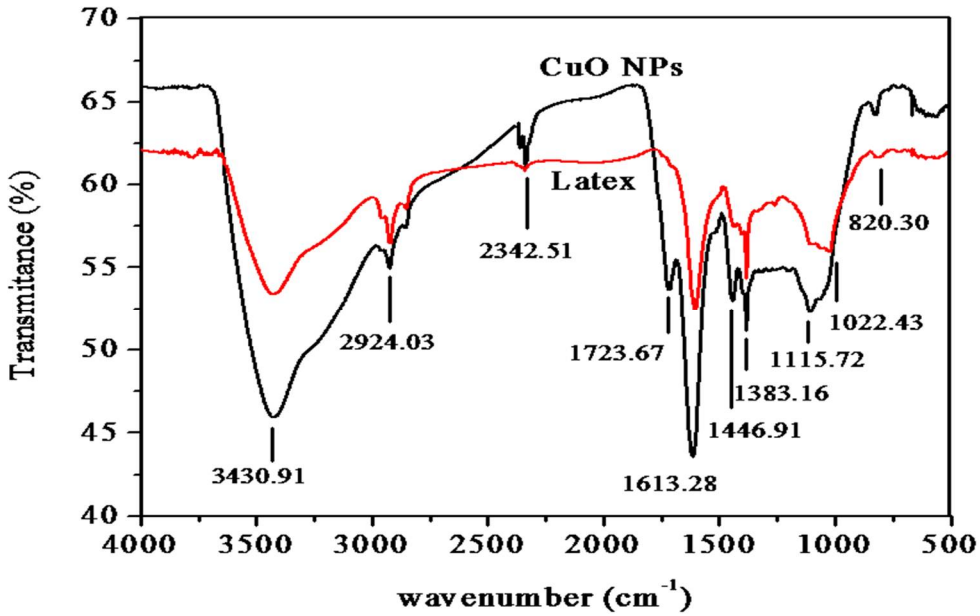

Figure. 3:Gives FTIR spectra of CuO NPs synthesized by using *Musa paradisiaca* latex

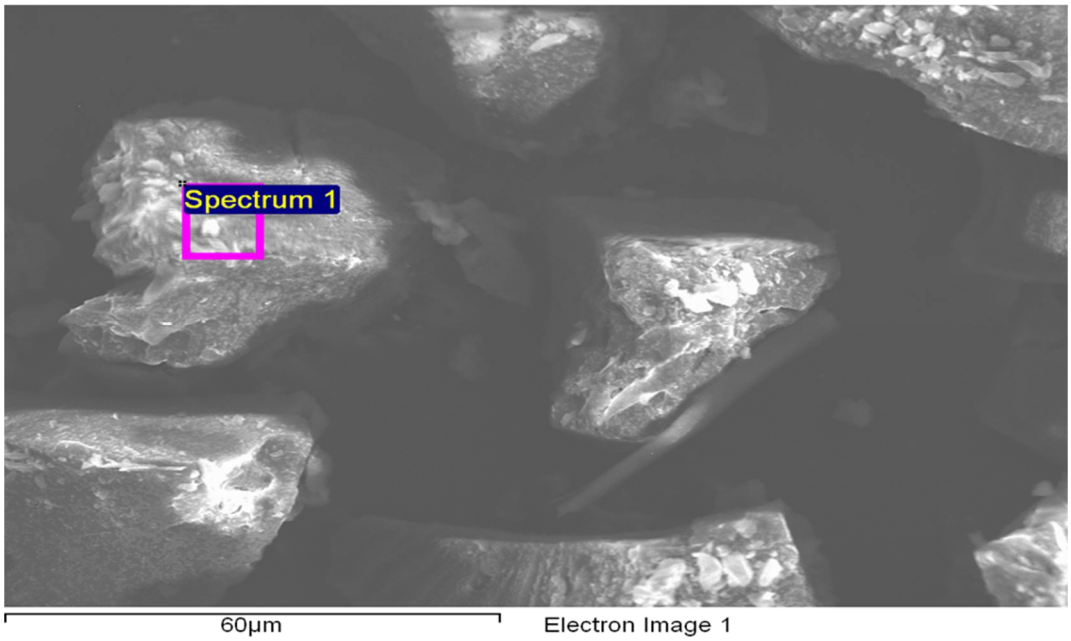

Figure. 4: Given the SEM images of synthesized CuO NPs

Scanning electron microscopy [SEM] and Transfer electron microscopy imaging analysis [TEM] techniques were carried out to confirm the size and morphology of the synthesized CuO NPs. The performance of CuO NPs was much influenced by size and morphological behavior and which are important parameters for investigation of nanoparticles. The typical SEM images of CuO nanoparticles synthesized from *Musa paradisiaca* latex extract as depicted in Figures 4 and 5, the morphology of the synthesized nanoparticles was observed roughly spherical shape and some aggregations are observed due to this SEM image is not clear. Further the size and shape of the synthesized CuO NPs was confirmed by HRTEM Analysis.

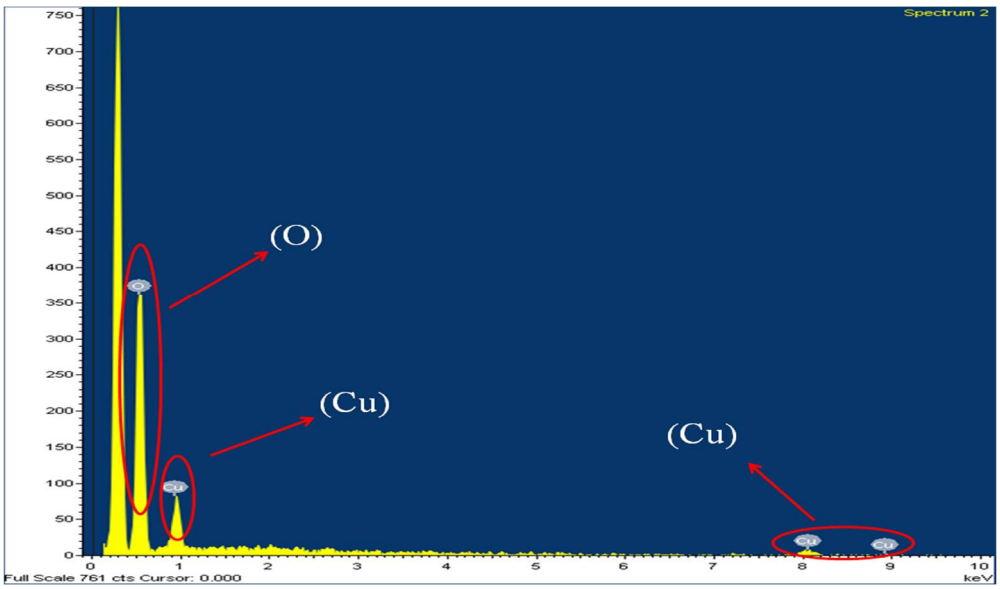

Figure. 5:Shows the SEM images of synthesized CuO NPs

The X-ray diffraction spectrum of synthesized CuO NPs is given in Figure. 6. The diffraction peaks observed at $2\theta^0$ values of 43.27, 50.37, 61.43 and 73.79 which can be assigned to (111), (200), (113) and (220) planes of FCC structure of pure copper NPs (International Centre for Diffraction Data (ICDD) no. 00-004-0836. Due to the presence of plant material around copper shell) additionally, there is few other diffraction peaks are appeared (36.37 and 42.02). After calcination (Figure. 6), when the oxides reduced appreciably and the intensity of diffraction peaks of CuO NPs significantly increased. The diffraction peaks at $2\theta^0$ of 32.40, 35.55, 38.68, 48.68, 53.49, 58.30, 61.65, 66.24, 68.12, 72.28 and 75.21 which can be assigned to (110), (002), (-111), (-202), (020), (202), (-113), (-311), (220), (311), and (-222) (ICDD no. 00-005-0661). In the XRD pattern, no other phases were observed [21], [46], [49],[50] &[51] and obtained diffraction peaks can be indexed as typical monoclinic in structure. From the figure 6, it is clear that the resultant particles are CuO nanoparticles and confirms by XRD study.

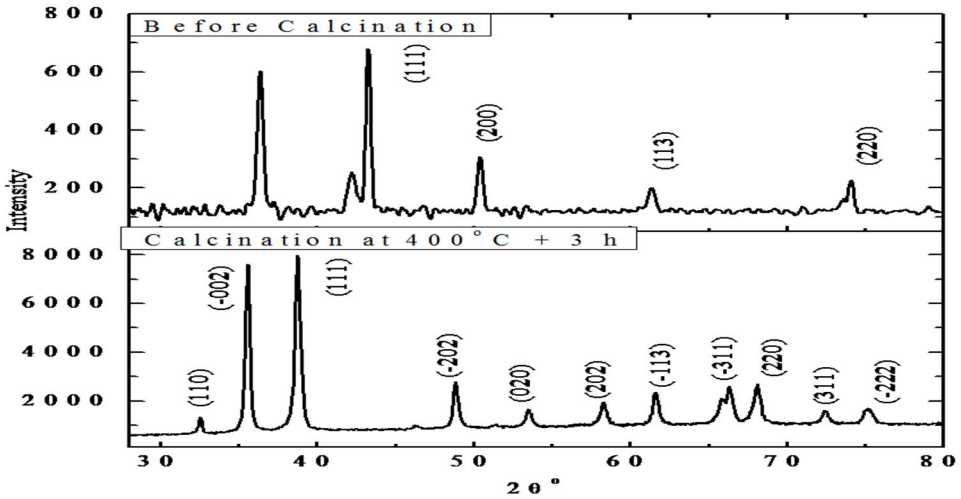

Figure. 6:Illustrates the XRD patterns of the synthesized CuO NPs
(a) **Before calcination (b) After calcination**

The elemental composition of synthesized sample was further confirmed using energy dispersive X-ray spectroscopy (EDAX) equipped with a scanning electron microscope. EDAX analysis gives the additional proof for the development of CuO nanoparticles through green synthesis with plant extract. Figure. 8 stand for the EDAX Spectrum of synthesizedCu oxide NPs. The EDAX results confirmed the presence of pure copper oxide nanoparticles at major emission energy of 1 keV and indicateno other elements were observed.

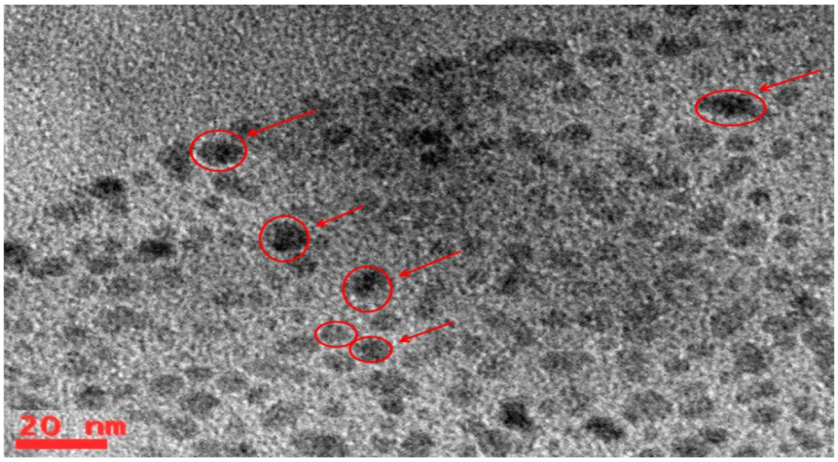

Figure. 7: Shows HR-TEM Image of synthesized CuO NPs

HR-Transmission electron Microscopy is the final confirmation and good argument for UV-Vis Spectrophotometer, XRD, and SEM results. Figure. 7contains the TEM images of prepared Cu oxide NPs, the synthesized CuO NPs are well dispersed spherical in shape and the average size of the CuO NPs is around 8 nm. Further these results were supports the particle size analyzer.

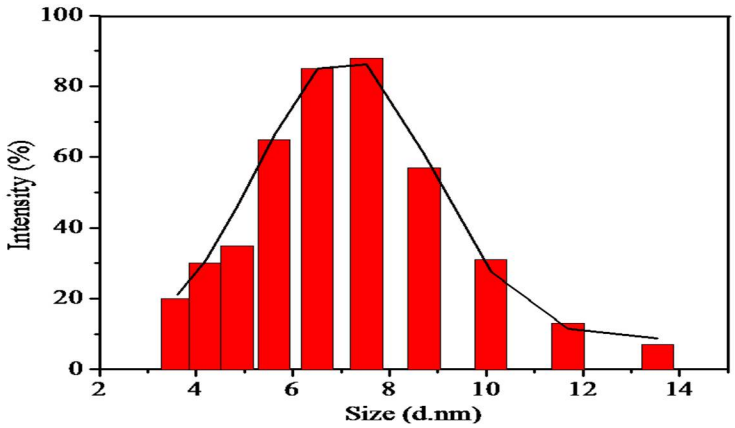

Figure. 8: Shows histogram particle size distribution of synthesized CuO NPs

The particle size distribution pattern results are displayed in Figure. 8. Here, particle size analyzer is used to measure the particle size distribution of synthesized CuO NPs. From the histogram, it is evident that most of the particles are in the range of 3-14 nm patterns. However, the majority of particles are ranging from 6-8 nm. It is further supports that the results showed in SEM and TEM studies.

Antimicrobial activity

Table1:Zone inhibition of CuO NPs forvarious microorganisms

S. No.	Tested Pathogens	Zone of inhibition (MM) CuO Nps				
		10 µl	20 µl	30 µl	Streptomycin	Latex
1.	*E.coli*	7	9	12	14	0
2.	*Staphylococcus aureus*	0	7	9	12	0
3.	*Pseudomonas aeruginosa*	0	0	7	11	0
4.	*Klebsiella*	0	0	0	9	0

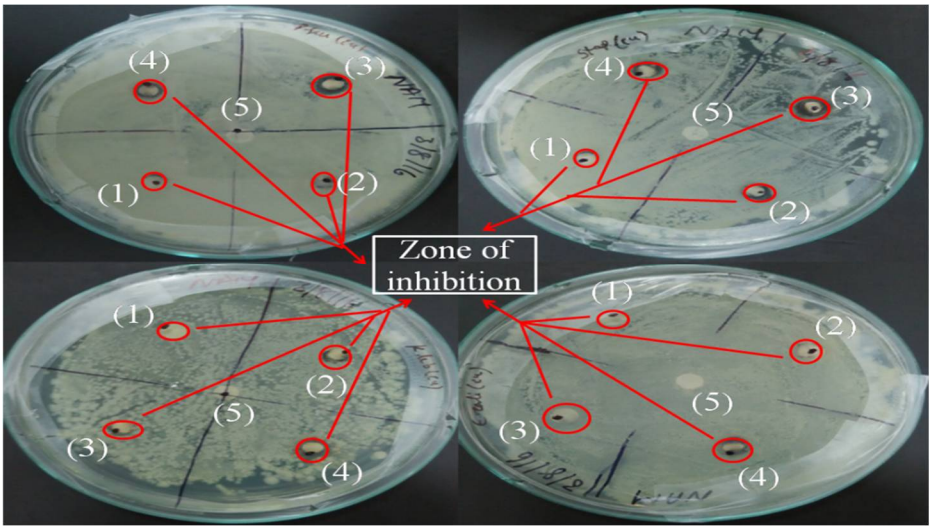

Figure. 9: Shows the antimicrobial activity of synthesized CuO NPs

The *Pseudomonas aeruginosa*, *Staphylococcus aureus, klebseilla* and *Escherichia coli* Sps bacteria's by disc diffusion testwere used for studies of antibacterial activity of the synthesized copper oxide nanoparticles using *Musa paradisiaca* latex extract.The diameter of the inhibition zones (mm) around each well with CuO NPs are represented in Table 1. Among this, when compared with standard antibiotic (streptomycin), the CuO NPs demonstrates a zone diameter of 12 mm at a concentration of 30 µL of CuO NPs, it is nearer to positive control (i.e. 14 mm zone of inhibition) and which is very similar to previous studies [53] also is observed highest zone of inhibition was observed on *Escherichia coli*. The ZnO NPs represent the antibacterial activity against *S. aureus* was reported by Raghupathi et al [54]. Figure. 9 shows the images antimicrobial activity of synthesized CuO NPs. The antibacterial activity of CuO NPs against both gram-positive and negative bacterial strains was reported by Azam et al. [55]. It was observed that the antibacterial test results showed that 10 µL concentrations of CuO NPs for all test pathogens is less susceptible to CuO NPs when compared to higher concentration of CuO NPs which is in agreement with the results reported in literature. Also similarly observed that Cu Nps of Nelumbo nucifera seed extract exhibited reliable antibacterial activity on Staphylococcus aureus (Gram-positive), Bacillus subtilis (Gram-positive), Pseudomonas aeruginosa (Gram-negative) and Escherichia coli (Gram-negative) [56]. Mubarak ali et al. reported that the bio-synthesized silver and gold nanoparticles are active against clinically isolated human pathogens, *Staphylococcus aureus* and *Escherichia coli* [57]. Ashavani et al. statemented that the silver-nanoparticles paint were coated on green-synthesized surfaces which showed excellent antimicrobial properties by killing both Gram-positive human pathogens (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) [58].

Possible Mechanism of the Antibacterial Activity of CuO Nanoparticles

It is known fact that the metal nanoparticles exhibit the strongest antibacterial effect against several bacteria's [59]. The exact mechanisms of antibacterial effect of metal nanoparticles are still not known, but it was reported that, two possible mechanisms could be involved in the interaction between nanoparticles and bacteria (i) the production of increased levels of reactive oxygen species (ROS), mostly hydroxyl radicals and singlet oxygenand (ii) deposition of the nanoparticles on the surface of bacteria or accumulation of nanoparticles either in the cytoplasm or in the peri plasmic region causing disruption of cellular function and/or disruption and disorganization of membranes [54]. We believe that both production of the ROS and accumulation or deposition of Cuo nanoparticles within the cytoplasm or on the surface of selected bacteria leads to either inhibition of bacterial growth or killing of bacterial cells [60]. Similar results were reported by sivaraj et al [52].

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CONCLUSIONS

In this research article, authors have developed an environmental friendly, hygienic, harmless and easy process for production of CuO nanoparticles using *musa paradisiaca* aqueous latex. Based on the

experimental findings, the UV-visible, IR and XRD analyses techniques were used for confirmation of formed CuO nanoparticles. The average size of 8 nm can be seen in SEM image of CuO nanoparticles. Latter spotlessness of biosynthesized copper oxide nanoparticles was confirmed by the occurrence of copper and oxygen elements and which clearly revealed via Energy-dispersive X-ray spectroscopy. The confirmed/synthesized CuO nanoparticles were highly steady, globular in shape around 8nm, and acts as a good antibacterial agent against *Pseudomonasaeruginosa*, *Staphylococcus aureus, Escherichia coli and klebseillaa* respectively. The reduction of copper sulphate to copper oxide nanoparticles was caused by the presence of biomolecules in agricultural waste extract. This developed nanoparticles of CuO were could be potentially used in are as such as food and medical applications.

Conflict of interest: We declare that we have no conflict of interest**.**

Human and animal rights: The research work of this paper has not involved any human participants and animals.

Informed consent: We have taken consent whichever is required in part of this paper

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