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Green Synthesis of Copper Nanoparticles using *Mitragyna parvifolia* Plant Bark Extract and Its Antimicrobial Study

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ABSTRACT

In this study, *Mitragyna parvifolia* plant bark used to prepare aqueous extract which provides cost-effective, eco-friendly process, less time consuming, an environmentally benign, easy and proficient way for the synthesis of copper nanoparticles. *Mitragyna parvifolia* plant bark was collected from Virpur hills forest area. The *Mitragyna parvifolia* plant bark extract was prepared with deionised water and used for the green synthesis of copper nanoparticles. The colour change of the solution dark brown from pale yellow colour confirms the formation of copper nanoparticles. The green synthesised copper nanoparticles were characterised by UV-visible spectroscopy, FT-IR, XRD, SEM, TEM and their antimicrobial activity was also investigated. UV-visible spectral result confirmed the reduction of copper sulphate to copper nanoparticles. FTIR analysis also supported for the formation of copper nanoparticles. Crystallinity of Cu NPs was found out by XRD study and the morphology of the particles was analysed with the help of scanning electron microscopy and found spherical in nature. The antibacterial activity experiment done against *Escherichia coli*, gram-negative and *Bacillus subtilis*, gram-positive bacteria by agar well method and the maximum zone of inhibition was higher in gram-positive bacteria compared to gram-negative bacteria. The green synthesised copper nanoparticles proved to be potential candidates for medical application where the antimicrobial activity is highly essential.

1. Introduction

Nanotechnology has involved many researchers from different field like chemistry, physics, biotechnology, material science, engineering, medicine, pharmaceutical etc. Copper nanoparticles synthesised by many ways such as biological method, chemical method, physical method, sol-gel method, solid-state reaction, co-precipitation, vapour deposition [1], electrochemical reduction [2], radiolysis reduction [3], thermal decomposition [4] and chemical reduction of copper metal salt [5]. Green synthesis method has so many advantages compared to other methods and one of the best methods because of its cost-effective, simple, use of low energy, use of less toxic materials and eco-friendly [6, 7]. The copper nanoparticles are mostly found their applications in the field of medical, electronic devices, biosensors, and reagents in various reactions, lubricants, antibiotic, antimicrobial agents and many more.

The *Mitragyna parvifolia* is a tree belongs to *Rubiaceae* family. This species are used medicinally and their height of 50 feet with a branch spread over 15 feet and a stem is erect and branching, flowers are yellow and grow in ball-shaped clusters, dark green in colour leaves smooth and round in shape [8]. The medicinal plant which contains a variety of phytopharmaceuticals found very important applications in the area of agriculture, human and veterinary medicine and novel drug for the treatment and prevention of disease [9]. The plant bark and roots are used in the treatment of fever, colic, muscular pain, burning sensation, poisoning, gynaecological disorders, cough, oedema and fruit juice augments the breast milk in lactating mothers [10].

Batool et al. [11] have prepared green synthesis copper nanoparticles using *Solanum lycopersicum* (tomato aqueous extract). In preparation of CuNPs used the chemical reduction method on different temperature and found average nanoparticle size 40-70 nm. Subhankari and Nayak [12] have synthesised copper nanoparticles by Ginger (*Zingiber officinale*) extract. They studied various microbiological testes with various concentrations of CuNPs with a size of 40 nm. Gopinath et al. [13] have synthesised copper nanoparticles from *Nerium oleander* leaf aqueous

extract and its antibacterial activity. They reported nanoparticles derivatives used for medical purposes like ulcer, infection and anti-bacterial pathogens. Khan et al. [14] have synthesised copper nanoparticles for a chemical reduction approach. The shape of the nanoparticle was cubic with their size of 28.73 nm and have great advantages of these NPs applied in catalysis and photo activated energy conversion. Kulkarni et al. [15] have synthesised copper nanoparticles using *Ocimum sanctum* leaf extract. Leaf extract was prepared in deionised water and these extracts added with 1 mM copper sulphate solution, the colour was changed which indicates the formation of CuNPs.

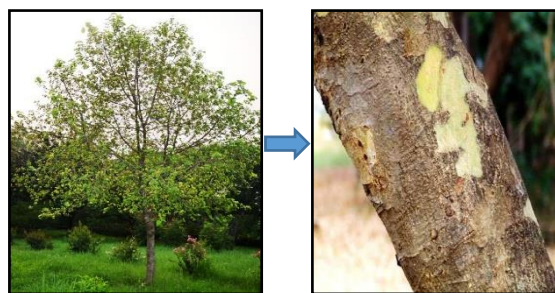


Fig. 1 Bark of *Mitragyna parvifolia* plant

Lee et al. [16] have a biological synthesis of copper nanoparticles using plant extract. In a high resolution of TEM analysis indicate a size of NPs 40-100 nm for the prepared particles by chemical and physical methods. It was given an application in various human body areas. Subhankari et al. [17] have synthesised of copper nanoparticles using *Syzygium aromaticum* (Cloves) aqueous extract by using green chemical reduction method and TEM reports revealed particle shape and size in the range 5-40 nm. These NPs had good stability. Angrasan and Subbaiya [18] studied biosynthesis of copper nanoparticles by *Vitis vinifera* leaf an aqueous extract and its antibacterial activity. By the reduction of copper sulphate into copper nanoparticles confirmed by UV spectra and IR which is obtained with strong band peak at 3431 cm⁻¹ and also shows good antimicrobial activity. *Staphylococcus aureus* exhibited the highest mean zone of inhibition.

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Kulkarni et al. [19] studied biosynthesis of copper nanoparticles using an aqueous extract of *Eucalyptus* sp. plant leaves. XRD data reveals the size of the nanoparticles as 23.18 nm. Jayalakshmi and Yogamoorthi [20] have a synthesised of copper oxide nanoparticles using *Cassia alata* flower extract and this copper oxide nanoparticles characterised and formation were identified by the colour change of the mixture from dark brown to pale green and showed a broad peak at 263 nm in UV-visible spectra and showed nano particle nature in SEM images with the range of size 120-280 nm. Awwad et al. [21] have synthesised copper nanoparticles using *Malva sylvestris* leaf extract and its antibacterial activity. The particle was crystalline in nature with 14 nm size and found that particles were highly stable and it gives significant effect on gram-negative and gram-positive bacteria.

Khodashenas and Ghorbani [22] have reported a synthesis of copper nanoparticles by various methods. Copper nanoparticles were examined by various methods like chemical, physical and biological methods. Given biological methods are cheaper, eco-friendly and easy for use. Varshney et al. [23] reported a biological synthesis of silver and copper nanoparticles. CuNPs are being fabricated globally with developing clean, non-toxic and eco-friendly technologies and fabricated spherical shape copper nanoparticles with size 4-10 nm. Manikanandan and Sathiyabama [24] studied copper nanoparticles synthesis and study of its antibacterial activity.

Saranyaadevi et al. [25] synthesised nanoparticles by using *Capparis zeylanica* leaf extract. The leaf extract act as capping and reducing agent and the colour change after addition of copper sulphate solution in extract. It was synthesised with cubical structure with a particle size between 50-100 nm. Lanje et al. [26] have synthesised and done optical characterization of copper nanoparticles. Hyungsoo et al. [27] have prepared seedless growth of free standing copper nanoparticles by chemical vapour deposition.

Hashemipour et al. [28] have investigated on synthesis and size control of copper nanoparticle via electro chemical and chemical reduction method. Mallikarjuna et al. [29] have green synthesis of silver nanoparticles using *Ocimum* leaf extract and their characterization. Nanoparticles resulted from its inhibitory activity against HIV-1, platelet aggregation and ADP22. Valli and Geetha [30] have synthesised copper nanoparticles using *Cassia auriculata* leave extract. The value of UV absorption obtained at 488.5 to 514.3 nm, SEM analysis got particle size range at 38.1 -43.5 nm and spherical shape and IR stretching frequency obtained at 509.21 cm^{-1} .

Molares et al. [31] have reported copper nanoparticles from tomato juice and they studied surface area and antibacterial activity. Copper nanoparticles are very attractive due to their unique properties. Takayama et al. [32] have reported copper nanoparticles keeps high surface area to volume ratio, low cost, catalytic activity, and optical properties and the main problem lie concerning preservation because they oxidized immediately in the air and reducing agents are very expensive and toxic [33-37]. Leopold and Xiong [38] have reported the plant extract and copper sulphate solution after mixing separate in 0.5 M, 0.1 M, 1.5 M and 2.0 M tomato juice were added to each flask then observe colour changed from no colour to yellow, orange, brown and finally, dark brown-black observed. Within a 15-20 min whole process was completed then the product was kept for 80 days and no sedimentation was observed.

Feldheim et al. [39] have reported nanoparticles have excellent inspiration and development of fundamental science. But generally, a problem with the physical and chemical method is the expensive, absorption of toxic chemicals and chemicals which are very hazardous will create so many side effects. Several methods like physical, biological, developed to prepare nanoparticles but unfortunately contain certain drawbacks [40]. Yeh et al. [41] prepared copper nanoparticles from the tomato extract by green synthesis method so this method very easy, cost-effective, and prepared in less time and mainly environmental friendly.

From the above literature it could be draft a conclusion that the green synthesis of metal and metal oxide nanoparticles are very cheap and ecofriendly with proficient qualities.

2. Experimental Methods

2.1 Material

The plant *Mitragyna parvifolia* was collected from Virpur hills and forest, Sabarkantha District, Gujarat. Ultra-pure deionised water used in the entire research. Analytical grade copper sulphate was purchased.

2.2 Preparation of *M. parvifolia* Bark Extract

Collected plant bark first washed with tap water and then again washed with double distilled water and dried it at room temperature for 15-20

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days. After dried, it was converting into powder form by using a grinder and collects it in neat and clean dry air tight bottle for use of research. About 10 g of powder took in 250 mL conical flask and added 100 mL deionised water and stirred for 30 min at 60 °C using magnetic stirrer. The extract was cooled down at room temperature and filtered with Whatman no.1 filter paper and the filtrate obtained was stored at room temperature at the dark place for further use.

2.3 Synthesis of Copper Nanoparticles

The reaction mixture was prepared by 10 mL of *M. parvifolia* extract was added to 90 mL of an aqueous copper sulphate solution in a 250 mL conical flask. After that, the solution colour changed pale yellow from blue when the solution of *M. parvifolia* extract and copper sulphate were stirred on the magnetic stirrer for a homogeneous mixture. After that, the flask was kept at room temperature for incubation around 24 hours and the colour was changed turn into dark brown from pale yellow (Fig. 2). The reaction mixture was centrifuged at 10,000 rpm for 15 min followed by dispersion of the pellet in deionised water and the CuNPs were dried in an oven at 80 °C for 4-5 hours [42].

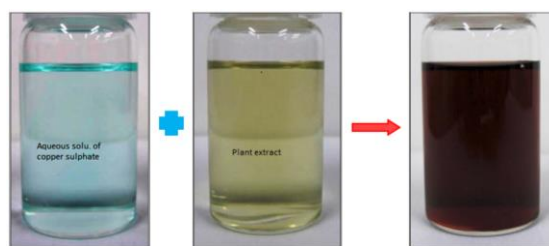


Fig. 2 Aqueous solu. of copper sulphate + plant extract = after synthesis of CuNPs

2.4 Characterization of Prepared Copper Nanoparticles

The green synthesized CuNPs characterisation was monitored by Shimadzu 1800 UV-visible spectrophotometer in the wavelength range of 300-700 nm. The Fourier transform infrared spectra were identified by an FT-IR spectrophotometer (Perkin Elmer Spectrum) using KBr. The sample powder was mixed with KBr and prepared pallet scanned at the range of 4000-450 cm^{-1} .

The X-ray diffraction (XRD Diffractometer (powder) Philips Xpert MPD Range (2 θ): 3° to 136°) was used to obtain the crystalline structure and data in the 2 θ range of 20°-80°. The Debye Scherrer's formula, $D = k\lambda / \beta \cos\theta$, where, D = particle diameter size, K = constant equals 1, λ = wavelength of X-ray source, β = the full width at half maximum of the diffraction peak and θ = the Bragg angle, was used to calculate the crystallite size.

The surface morphology of CuNPs was analyzed by scanning electron microscope, performed by SIGMA model. TEM analysis using Ransmission Electron Microscope (TEM) With CCD Camera, Model: Tecnai 20, Make: Philips, Holland, used to character the size, shape and morphology of the copper nanoparticles. A thin sample was irradiated with a sharp high-energy electron beam focused by magnetic lance and electron intensity distribute on the beam after interaction with sample and image was recorded by digital camera and display on a computer screen.

2.5 Antimicrobial Activity

2.5.1 Test Organism for Antibacterial Activity

In this study, two type bacteria were collected from the microbiology department, HNGU. One was gram-positive bacteria, *Bacillus subtilis* (+ve) and another one was gram-negative, *Escherichia coli* (-ve). The bacterial strains were grown and maintained on nutrient agar at 38 °C in incubation condition for 5 days and the culture was stored at 4 °C for further experiment work.

2.5.2 Media Preparation

In the media preparation, *B. subtilis* and *E. coli* bacteria were grow in a nutrient agar medium. 2.8 g nutrient agar powder was added into 100 mL of distilled water for nutrient agar preparation. Then the prepared medium was kept in the cotton-plugged glass container and sterilized in the autoclave at 120 °C for 20 min.

2.5.3 Method for testing Antimicrobial Activity of Synthesised Copper Nanoparticles

Antimicrobial activity of green synthesised copper nanoparticles was carried out by agar disc diffusion method [43-45] against *B. subtilis* (+ve) and *E. coli* (-ve) bacteria.

The nutrient agar plates were prepared by 20 mL for each of molten media into sterile Petri-plates. Plates were left standing for 10 minutes to let the culture get absorbed.

Using the micro-pipette, 100 μL of the sample of nanoparticle suspension was poured into different concentration (25 μL , 50 μL , 75 μL) into each plate. Then antibiotic-ampicillin drug was used as positive control. After adding the samples in the wells, the dishes were kept in a refrigerator for an hour for absorption of the samples into the surrounding medium from the well. The plates were transferred into an incubator set at 37 °C to allow bacterial growth on the medium. After 24 hours the plates were taken out of the incubator and observed for the zone of bacterial growth inhibition around the wells. The zone of inhibition was measured in millimeters [46].

3. Results and Discussion

3.1 UV-Visible Spectroscopy

The characterization of copper nanoparticles by UV-vis spectra from the range of 500 - 750 nm provide clear absorption peaks. The UV-Vis spectra reveals the absorption by the CuO nanoparticles obtained at 565-570 nm as shown in Fig. 3. The brown color indicated the formation of copper nanoparticles in water (Fig. 2). In Fig. 3 shows UV-Vis spectroscopy absorption peak would be obtained around 565-570 nm [47].

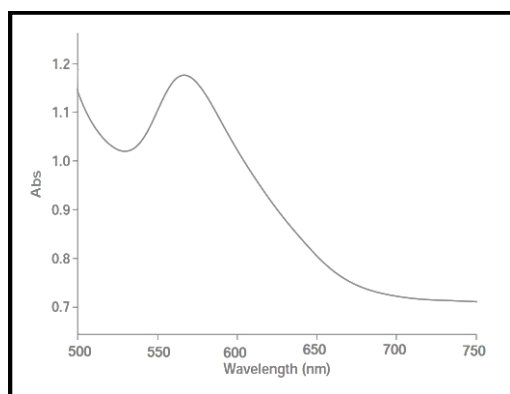


Fig. 3 UV-visible spectra of copper nanoparticles

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR gives the information about the vibrational absorption present in synthesised copper nanoparticles and it shows in Fig. 4. In the spectra the peak at 3298.28 cm^{-1} and 3324.21 cm^{-1} indicating the presence of -OH group stretching in acids, alcohols and phenols, stretching at 2926.01 cm^{-1} corresponds to C-H stretching in alkanes and aldehydes, stretching at 1648.12 cm^{-1} indicate the presence of >C=O group and the peak at 1103.28 cm^{-1} corresponds to C-O stretching and the weak peaks inbetween 850.61 cm^{-1} to 526.57 cm^{-1} are associated to halo compounds stretching [48, 49]. Hence these observations indicated the formation of CuNPs associated with metabolites like terpenoids contain functional groups as alcohols, phenols, aldehydes, ketones and carboxylic acids. Kulkarni et al. reported the bio-entities could probably play a double role of fabrication and stabilization of copper nanoparticles in the aqueous solution [50].

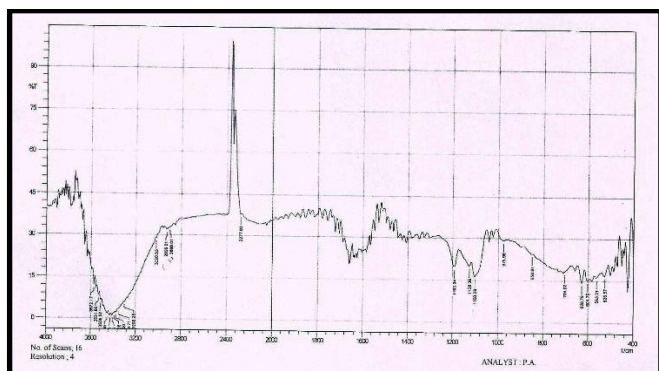


Fig. 4 FTIR spectra of copper nanoparticles

3.3 X-Ray Diffraction

In the X-ray diffraction study, the peaks observed 2θ value at 43.31°, 50.55° and 74.27° representing the (111), (200), and (220) planes (Fig. 5). <https://doi.org/10.30799/jnst.133.18040415>

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Morphology of the interplanar distance spacing was calculated by using Bragg's equation. Morphology of the interplanar distance spacing was calculated by using the Debye Scherrer's formula, $D = k\lambda / \beta \cos\theta$, where, D = particle diameter size, K = constant equals 1, λ = wavelength of X-ray source, β = the full width at half maximum of the diffraction peak and θ = the Bragg angle. The size of the NPs obtained were estimated to be 27.45 nm using Debye- Scherrer's equation, which may indicate a high surface area, and surface area to volume ratio of the nano-crystals.

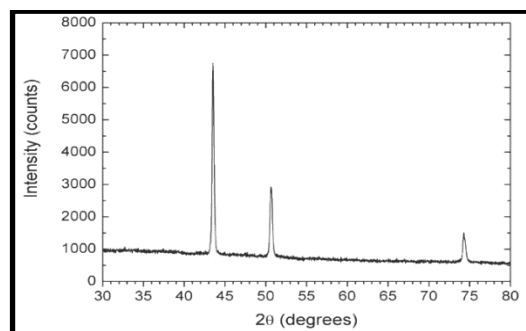


Fig. 5 X-ray diffraction pattern of copper nanoparticles

3.4 Scanning Electron Microscopy (SEM) Analysis

The copper nanoparticles morphology determined by scanning electron microscope image. From the surface morphology study, the size of Cu NPs obtained as 23.6-41.2 nm. Fig. 6 shows the existence of symmetrical spherical shape of Cu NPs. The electronic interaction and hydrogen bond between the bio-organic molecules bond are responsible for the synthesis of copper nanoparticles using plant extract [51].

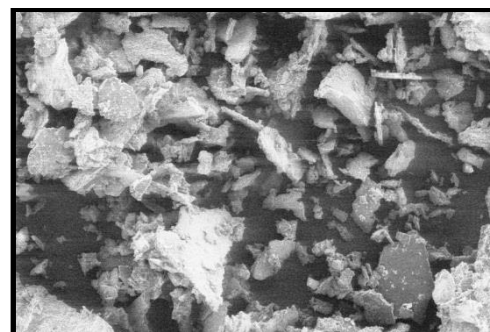


Fig. 6 SEM analysis of copper nanoparticles

3.5 Transmission Electron Microscopy (TEM) analysis

The image of silver nanoparticles synthesised using an aqueous extract of (plant name) shown in Fig. 7. The synthesised Cu NPs was spherical in shape and an average diameter of 12-23 nm. Singh et al. have reported a similar geometry of synthesized silver and gold nanoparticles using natural precursor clove [52].



Fig. 7 TEM analysis of copper nanoparticles

3.6 Antimicrobial Activity

The antimicrobial activity of green synthesised copper nanoparticles against two human pathogenic bacteria such as *Bacillus subtilis* and *Escherichia coli*. Here *Bacillus subtilis* is gram +ve and *Escherichia coli* is gram -ve bacteria were evaluated and compared to a commercial antibiotic drug ampicillin. Synthesised CuNPs showed the clear diameter

of the zone of inhibition around the well wherein the suspension of CuNPs was applied. The obtained result was presented in Table 1 and Fig. 8.

Table 1 The Zone of inhibition area (mm) exhibited by the formed CuNPs against pathogenic bacteria

Concentration (μL)	Diameter of zone of inhibition (mm)	
	<i>Escherichia Coli</i>	<i>Bacillus Subtilis</i>
25	6.80	9.50
50	10.00	13.80
75	13.80	17.50
Standard Drug Ampicillin	16.00	20.00

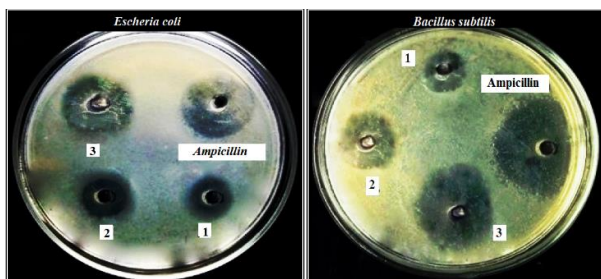


Fig. 8 The zone of inhibition of green synthesis of Cu NPs against pathogenic bacteria

4. Conclusion

In this study, green synthesis of copper nanoparticles was successfully synthesised using aqueous extract of *Mitragyna parvifolia* plant bark which provides cost-effective, eco-friendly process, less time consuming, an environmentally benign, easy and proficient way for the synthesis of copper nanoparticles. From UV-visible spectral result it was confirmed the reduction of copper sulphate to copper nanoparticles. FTIR analysis also supported for the formation of copper nanoparticles. Crystallinity of Cu NPs was found out by XRD study and the morphology of the particles was analysed with the help of scanning electron microscopy and it was found with an average size of 23.6–41.2 nm. The antibacterial activity for the synthesised copper nanoparticles was confirmed by the antibacterial activity experiment against *Escherichia coli* gram-negative and *Bacillus subtilis* gram-positive bacteria by agar well method and the maximum zone of inhibition was higher in gram-positive bacteria compared to gram-negative bacteria. The green synthesised copper nanoparticles proved to be potential candidates for medical application where antimicrobial activity is highly essential.

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