Biological Forum – An International Journal **15(5): 1610-1615(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Macaranga indica Plant Assisted Rapid Synthesis of Copper Nanoparticles for Biomedical Applications

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(Received: 20 March 2023; Revised: 14 April 2023; Accepted: 22 April 2023; Published: 20 May 2023)
(Published by Research Trend)

ABSTRACT: Rapid synthesis of stable copper nanoparticles is a challenging process in the synthetic world. Here in this study, the above challenge was achieved via the green route by using *Macaranga indica* plant extract. *Macaranga indica* is a medicinal plant widely available in the western ghat evergreen forest. Since a decade ago, villagers of the western ghat belt have been using its medicinal properties like antibacterial, antioxidant, antidiabetic, and antidysentery as household remedies. In this current study, we have used its bark extract to prepare stable copper nanoparticles to increase its medicinal properties. Synthesized nanoparticles are characterized by using analytical tools like UV-Vis spectra, XRD, TG, FESEM, EDX, and TEM analysis. These techniques reveal that synthesized nanoparticles are crystalline monodispersed in nature and have a size of ~20-22 nm. Furthermore, these biocapped nanoparticles have shown good antibacterial and antioxidant properties and can be used for biomedical applications as well as in drinking water treatment.

Keywords: Rapid green synthesis, Copper Nano Particles, *Macaranga indica*, Microwave Assisted, Biomedical applications.

INTRODUCTION

Nanotechnology is a young, multidisciplinary field with roots in the physical, chemical, and biological sciences. The antibacterial and antioxidant characteristics of metal nanoparticles, such as copper (Cu), silver (Ag), and gold (Au), which rely on their creation history, have been thoroughly explored (Arya *et al*., 2018; Perumal *et al*., 2021; Ramyadevi *et al*., 2012). The optimum method for these nanoparticles' biological analyses is plant-assisted rapid green synthesis. For instance, plant extracts that function as reducing agents for metal ions also serve as bio-capping agents for metal nanoparticles and enhance biological activity. The remarkable biocompatibility and inherent antibacterial qualities of metal NPs have sparked a renewed interest in their potential use in biological applications (Que & Chang 2010; Ramyadevi *et al*., 2012).

The small to medium-sized *Macaranga indica* tree has smooth, grey bark and sturdy branchlets that are covered in leaf scars. The plant, which may grow up to 20 meters tall, is also known as *Macaranga flexuosa* wight in botany. In several Indian languages, the plant goes by numerous names. The plant's Malayalam name is "Puthata-mara," while its Kannada name is "Bettadavare." The plant is referred to as "Vattathamarei" and "Vuttuttamara" by Tamil-speaking people. Native to India, *Macaranga indica* is found both south and east of the country.

The leaves have a big, long petiole and are orbicularly oval, acuminate, whole, and extensively peltate. Dioecious, axillary panicles bear pale yellow flowers that are all dioecious. It can be found in Bangladesh's hilly region (Rana & Rana 2014). In addition, the plant can be found in China, India, Sri Lanka, Bhutan, and Myanmar (Ahmed, 1999). The plant's red gum is applied to sores (Esha *et al*., 2012) and used to clean wounds (Rana & Rana 2014). Numerous traditional medicines use various plant parts on a regular basis. The herb is used to cure tumors, paralysis, and anemia (Esha *et al*., 2012). Additionally, it is used to cure stomachaches (Esha *et al*., 2012), cuts, wounds, and venereal sores (Rana & Rana 2014). Occasionally, a gum secreted by the plant's fruits, young shoots, petiole bases, and cut branches is administered topically to treat venereal sores. Various illnesses are also treated using the plant's other parts in various ways (Jain, 2004).

Here, microwave irradiation is used to quickly synthesize CuNPs while also assisting in the removal of plant extract's enzymes. The created CuNPs can be kept at room temperature and are long-lasting in colloidal form (RT). CuNPs can be synthesized in a short time using this technology instead of a chemical/physical approach. It takes about six minutes for 80 percent of Cu^{2+} ions to be converted to CuNPs. In our previous work, we established the preparation of silver nanoparticles with *Macaranga indica* plant extract and

examined them for various biomedical applications (Hegde *et al*, 2023).

MATERIALS AND METHODS

Fresh *Macaranga indica* (*M. indica*) Stem Bark was collected in and around Western Ghats, Karnataka, India. Hi-media supplied analytical grade cupric nitrate, methanol, ethanol, and acetone (Bangalore, India). A microwave oven (LG MJEN326UH, 2.45 GHz) was used to prepare plant extract and irradiate reactants.

A. Synthesis of Copper Nanoparticles

A 10 g of freshly picked *M. indica* barks were cleaned with distilled water, removed foreign particles were, chopped into smaller pieces, and immersed in a beaker with 100 ml of distilled water. This was irradiated with 2.45 GHz microwaves for about 5 minutes to remove the phytoconstituents from the plant material. It was filtered through a 0.45 µm membrane filter to get rid of the fibers while it was hot. A 10 ml of this stock preparation was mixed with 40 ml of 103 M $Cu(NO_3)_2$ aqueous solutions. This mixture was treated in a microwave oven and heated for different amounts of time. Spectra (Shimadzu, 1650PC) for surface plasmon resonance (SPR) peak was used to keep an eye on the formation of CuNPs. Also, the amount of SPR peak absorption is used to measure the percent reduction of CuNPs. We waited 8 minutes for the 1 mM $Cu(NO_3)_2$ solution to be completely reduced, and then we looked at the absorption value (0.96 at 535 nm) of the SPR peak. This coefficient of absorption or extinction was taken as a reduction of 100%. After 8 minutes, there was no more increase in absorption, and the reaction mixture didn't change color. The metal NPs were then dried in a vacuum oven for roughly 12 hours at 70 °C after being rinsed many times with distilled water.

B. Characterisation of Synthesised NPs

UV-Vis Spectroscopy. This technique refers to the absorption of UV−Vis. radiation by the samples. UV−Vis. spectrum is simply a plot of absorption intensity versus wavelength. Beer-Lambert's law is very important here which states that the concentration of a substance and the thickness (path length) of a sample is directly proportional to the absorbance. The progress of the reaction is monitored by using a UV-Vis spectrophotometer (Systronics, 2207). The equipment was run at a 10 mm optical path length, and a 300 nm/min scan rate. 3 ml of the reaction mixture was periodically taken out into a quartz cuvette for analytical and optical absorption tests.

XRD technique. XRD technique is one of the versatile, powerful, and non-destructive methods to know the crystallographic information of samples. The XRD is governed by Brags condition: $n\lambda = 2d_{(hkl)} \sin\theta$. All crystalline materials produce distinct XRD patterns, which can be indexed for the identification of phases depending on the positions of peaks and their relative intensities. The powder XRD pattern of synthesized NPs was recorded using the 'X'PERT-PRO XRPD (Cu K) at a voltage of 40 kV and a beam current of 30 mA, with a scanning rate of 2° min1 and a temperature range of 10 to 80 °C. Prior to the experiment, the NPs were dried at 70–80°C in a vacuum oven.

TG analysis. In TG analysis, the sample's weight is calculated based on the temperature in scanning mode or the time in isothermal mode. It analyses the kinetics of physicochemical processes in the sample and characterizes decomposition and thermal degradation/stability. The excremental conditions used determine the mass change characteristics of the materials. The sort of information needed about the sample will determine the temperature program to use. The environment that is utilized or TG analysis also has a significant impact; the analysis can be done with either nitrogen (N_2) or argon as the atmosphere.

The heat degradation of bioactive compounds in these bio-capped NPs was investigated by TG analysis (Linseis STA PT1600, Germany). The experiment was conducted in a platinum crucible with N2 as the environment and a heating rate of 10°C min. We measured the weight loss NPs from room temperature to 80°C.

FESEM/EDX analysis. FESEM uses a concentrated beam of electrons to produce an image of a sample. The sample is exposed to an extremely high-energy electron beam produced by an electron gun. Inelastic or elastic scattering in the atoms at the sample surface is caused by these incident electrons, and electrons are ejected from the sample. Backscattered electrons are electrons ejected by an elastic collision between an incident electron and the atomic nucleus of a material. It is known as secondary electrons, and they are created by collisions with the nucleus that result in large energy loss, or by weakly bound electrons being ejected.FESEM (FEI Nova nano 600, Netherlands) and EDX spectra were used to describe the surface morphology of synthetic NPs. An ultrasonic bath was used to disperse the powder sample in acetone for FESEM examination. An aluminium stub with carbon tape attached to it was coated with a drop of this powder suspension. The scattered powder on the stub is left behind after the acetone evaporates from the stub's surface.

TEM analysis. An electron beam is delivered through an incredibly thin sample using the TEM electron microscopy technique, interacting with the material as it does so. The interaction of the electrons with the sample they are passing through results in the formation of a picture. A TEM (JEOL JEM 3010, Japan) is used to examine the sample's surface morphology. A drop of the dispersed sample in acetone was applied similarly to a copper grid coated with carbon to prepare the sample, allowing the solvent to evaporate. A voltage of 80 kV was used to accelerate the device.

C. Antibacterial and Antioxidant properties

The agar disk diffusion method is used to test the antimicrobial activities of NPs formed due to the binding of *M. Indica* bark extract. The mixture of yeast extract (5 g), peptone (8 g), agar (20 g,) and NaCl (12 g) were taken in a flask containing 950 ml of distilled water. The solution was mechanically stirred to get a clear media. Then the pH was adjusted to 6.8 by adding

sodium hydroxide solution. Then the total volume of the mixture/media is diluted to 1000 ml and kept for sterilization in an autoclave at 120°C for 20 min. The antioxidant scavenging activity of NPs is established by using the DDPH method. The prepared NPs were dissolved in methyl alcohol (10–100 µg/mL) and then put into various test tubes with 3 mL of methyl alcohol added to each. 2 ml of 0.005% DPPH in methanol was added to it and was then incubated at room temperature for about half an hour in the dark. The scavenging activity of DPPH was assessed using the absorbance at 516 nm.

RESULTS AND DISCUSSION

In Fig. 1a, the bark of *M. Indica* is depicted through photography. To extract the solution, the stem bark is treated with deionized water and chopped into tiny (1 cm) pieces. The mixture of *M.Indica* bark extract and Copper nitrate initial and final irradiation with microwave (MW) is shown in Fig. 1 b. The first hue of the copper nitrate and *M. Indica* bark extract mixture was a pale yellow. After eight minutes of microwave exposure, the colour change of the solution progressively varied to dark brown. Given that Cu^0 particles may produce a hue like this, this proves that Cu^{2+} has been reduced to Cu^0 (in contrast to irradiating plant extract alone). With increasing exposure time, the color intensity went from light yellow at t = 0 minutes to brown at t = 8 minutes (time given for 100 percent reduction).

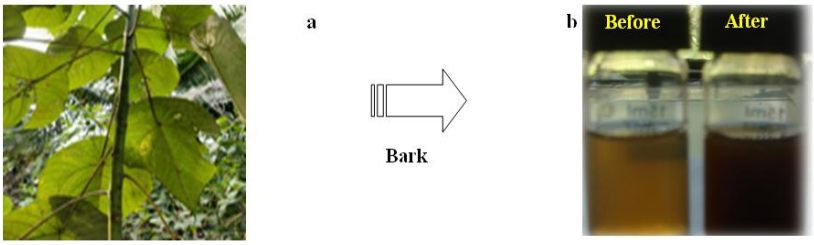

Fig. 1. (a) Photographs of *M. indica* Bark and **(b)** Extract of *M. indica* bark upon combining with $Cu(NO_3)_2$ solution before and after irradiation with MW.

Fig. 2 displays the Spectral plot of the reaction mixture's UV-Vis range after various microwave exposure times (0–8 min). The SPR of Cu^0 particles generated here is confidently attributed to the absorption peak maxima at 535 nm (Bhagat *et al.*, 2021). As anticipated, the absorbance rose as the reaction time rose. Additionally, we observe a blue SPR shift from 535 nm to 520 nm, which is most likely caused by an increase in particle size (Mott *et al.*, 2007). According to reports, CuNPs' absorption bands fall between 500 and 650 nm (Sathyavathi *et al*., 2010). The reduction reaction is brought on by the biomolecules in the extract of *M. indica* bark. However, the absorption increased around seven times when Cu^{2+} ions were mixed with the plant extract, and the SPR peak was clearly visible at 536 nm.

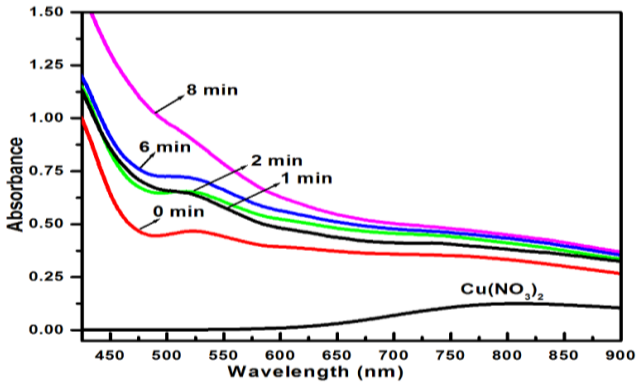

Fig. 2. The SPR peak for CuNPs can be seen in the UV-vis spectra of the reactant mixture (0-8 min).

The XRD pattern of the CuNPs is shown in Fig. 3, both before and after drying to 70 °C. Due to their amorphous nature and bio-capability, the first batch of CuNPs showed a diffuse pattern (Fig. 3a). The face-cantered cubic structure of copper can be seen in the XRD pattern (Fig. 3 b) at 2 = 43.3, 50.4, and 74.10, which correspond to (111), (200), and (220). (JCPDS No. 851326). CuNPs were found to be devoid of CuO, Cu_2O, Cu $(OH)_2$, and other contaminants (Yu *et al.*, 2009; Yunita *et al*., 2020).

Fig. 4 displays EDX spectra and FESEM images of bicapped CuNPs. The almost spherical-shaped particles (between 20 and 30 nm) are clearly visible in the FESEM image. Cu is the only significant element, according to the EDX spectrum. There are pollutants made up of the elements C and O everywhere. However, we have found larger concentrations of C (20.2%) and O (32.1%), this must be brought on by the phytochemical constituents present in the plant extract (Thakore *et al.*, 2019). As a result, we have used these elements as proof that the biological materials connected to the CuNPs exist. The TEM images biosynthesize CuNPs are displayed in Fig. 5. The TEM images unmistakably demonstrate the particles'

spherical shape and narrow size distribution, which have a diameter in the 20–25 nm range. Similar types of results are obtained by silver nanoparticles by using *Macaranga indica* plant extracts (Hegde *et al*., 2023).

The TG curve for produced CuNPs is shown in Fig. 6 under N2 atmospheric conditions. The adsorbed water molecules are responsible for the first weight loss of about 4% from room temperature to 100 °C. The degrading plant residue, or the bio-capping substance on the NPs, is blamed for the following weight loss of 14% from 100 to 550°C (Kasthuri *et al*., 2009). Following that, the weight loss is insignificant up to 80°C, and the CuNPs were preserved without oxidation due to the Nitrogen atmosphere.

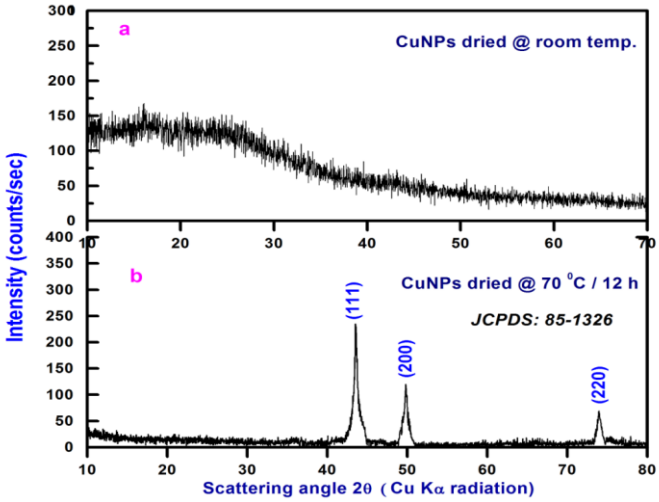

Fig. 3. XRD pattern of Synthesised NPs. a) at room temperature b) at 70°C.

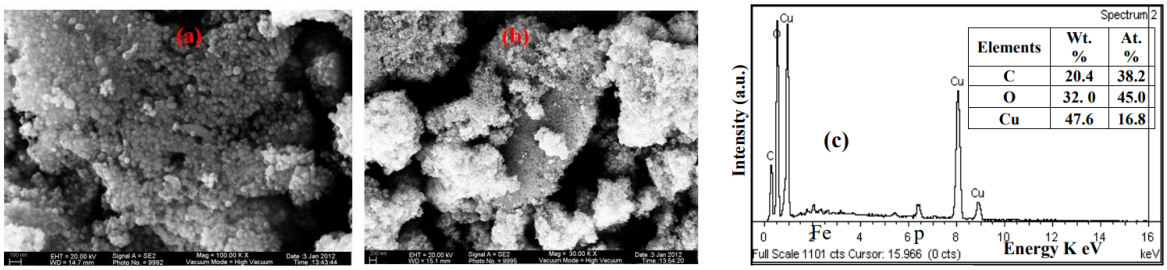

Fig. 4. (a, b) FESEM images and **(c)** EDX pattern of CuNPs.

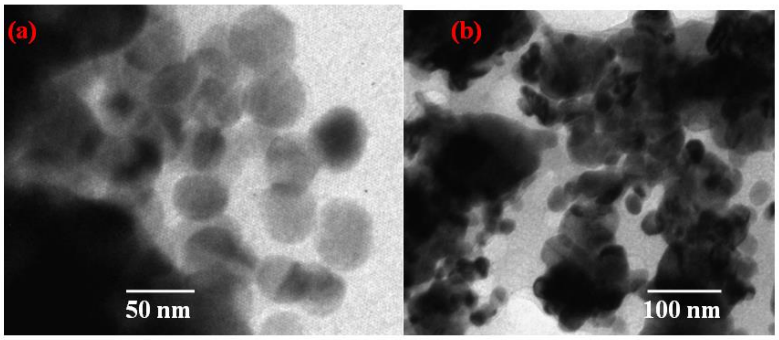

Fig. 5. TEM images of CuNPs.

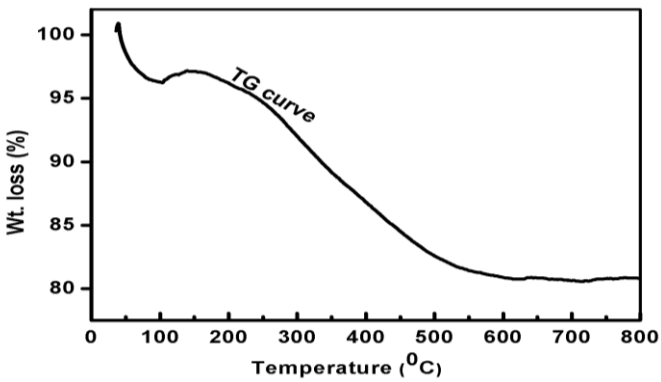

Fig. 6. TG curve for as-produced CuNPs.

Antibacterial and Antioxidant Properties. The disc diffusion method is used to assess the antimicrobial activity of CuNPs made with *M. indica* bark extract. Depending on the volume used against tested human pathogens, NP activity varies. Higher CuNP concentrations resulted in higher antimicrobial activities: 100% (10 g/ml) > 75% (7.5 g/ml) > 50% (5.0 g/mL). As CuNP concentration rises, the zone of inhibition expands (Valodkar *et al*., 2011). Table 1 provides a summary of CuNPs' antibacterial properties. The antibacterial zone inhibition activity of CuNPs dried at RT (a) was higher than that of CuNPs dried at 75 °C before and after the removal of biomolecules, though only marginally. In comparison to *P. aeruginosa* and *S. typhi*, the antibacterial activity of the biocapped CuNPs was higher against *E. coli* and *S. aureus*. Against *E. coli* cells, the biocapped CuNPs displayed greater antibacterial activity (Nicolaides *et al*., 2020; Valodkar *et al.*, 2011). Similar types of antibacterial activities of copper nanoparticles were observed in copper sulfate-doped *Ficus auriculata* lour. Fig. 7 depicts a typical photographic image of E. coli cell growth inhibition by CuNPs dried at room temperature (a), CuNPs dried at 75 °C (b), CuNPs washed with methyl alcohol/water and dried at 75 °C (c), and the drug ciprofloxacin (d) Experiment results also support the effectiveness of CuNPs as antifungal agents**.**

Table 1: CuNPs' antimicrobial activity as measured by disc diffusion.

Bacteria	Zone of inhibition (mm)				
	100%	75%	50%	Ciprofloxacin (std.)	Control 10% DMSO
(a) CuNPs dried at RT					
S. aureus	12	08	07	16	-
S. typhi	11	08	05	20	-
E. coli	17	15	12	21	-
P. aeruginosa	09	06	05	19	-
(b) CuNPs dried at 75 °C					
S. aureus	11	09	06	16	-
S. typhi	08	06	05	20	-
E. coli	15	11	10	21	-
P. aeruginosa	07	05	03	19	-
(c) CuNPs washed with methyl alcohol/water and dried at 75 °C					
S. aureus	08	06	05	16	-
S. typhi	07	06	05	20	-
E. coli	08	06	05	20	-
P. aeruginosa	05	01	-	19	-

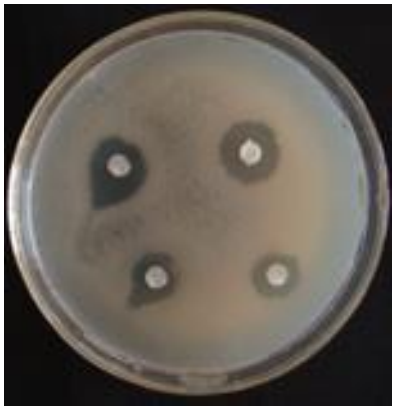

Fig. 7. A typical photographic image showing the growth inhibition of *E. coli* cells for **(a)** dried at RT, **(b)** CuNPs dried at 75 °C,**(c)** CuNPs washed with methyl alcohol/water and dried at 75 °C and **(d)** ciprofloxacin drug.

The stable nitrogen-centered free radical DPPH undergoes a color change from purple to yellow upon reduction and exhibits a distinctive absorption at ~518 nm(Chien *et al.*, 2013) . Antioxidants and DPPH interact to change DPPH's color and transform it into 1,1-diphenyl-2-picrylhydrazine. The antioxidant qualities of CuNPs are comparable to those of ascorbic acid as shown in Fig. 8. This is because CuNPs are oxidized quickly and/or in the right amount (Cu^0+DPPH→Cu^{2+}+1,1–diphenyl–2–picryl hydrazine). Here CuNPs inhibited the activity of DPPH by donating its electron.

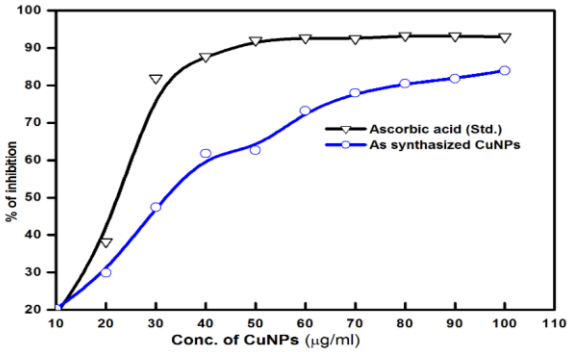

Fig. 8. The antioxidant capacity of copper nanoparticles compared to that of standard ascorbic acid.

CONCLUSIONS

In this study, employing *M. Indica* plant bark extract act as a reducing and stabilizing agent, the biocapped CuNPs of almost spherical form were successfully produced using microwave irradiation. The UV-Vis spectra for the SPR peak, 535 nm, are used to track the synthesis of CuNPs. With rising reaction mixture temperature, SPR intensity rose linearly. The marginal

pH reduction and E increase of the reaction mixture provide evidence in favor of the CuNPs' production mechanism. According to UV-Vis spectra, the highest reduction, which converted 80% of the Cu^{2+} ions into CuNPs, occurred in less than 6 minutes, while the full reduction occurred in roughly 8 minutes. These CuNPs' structural and microstructural information was gathered utilizing the techniques of XRD, EDX, TG, and FESEM/TEM. The CuNPs' XRD pattern and reported statistics for copper metal coincided, and the crystal size is around 23 nm. The heat breakdown of plant waste materials and the oxidation of Cu to CuO are demonstrated by TG analysis. The homogeneous spherical particles created here are visible in FESEM/TEM pictures. As anticipated, the presence of Cu alone is confirmed by elemental analysis by EDX. The synthesized *Macaranga indica* bark extract-mediated copper nanoparticles have shown good antimicrobial properties and antioxidant properties compared with the standard drugs available in the market. Hence, this can be used in the biomedical field as a potent drug.

FUTURE SCOPE

The rapid green synthesis route explained above may help to produce environmentally friendly NPs particles by using the *Macaranga indica* plant extract which reduces the energy of production and environmental pollution. Their ability to inhibit the growth of microbial colonies may lead to their usage in drinking water purification and treatment of wastewater. Furthermore, this can be used for various other biomedical applications like disinfectant preparation, preparation of sanitizers, floor cleaners, and as surface coating agents.

Acknowledgment. Authors thank the Department of Chemistry, Dr. A.P.J. Abdul Kalam University, for carrying out this research work.
Conflict of Interest. None.

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How to cite this article: Gajanan Hegde and Tanuja Kadre (2023). *Macaranga indica* Plant Assisted Rapid Synthesis of Copper Nanoparticles for Biomedical Applications. *Biological Forum – An International Journal*, *15*(5): 1610-1615.