BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, March 2023. Vol. **20**(1), p. 317-328

Anti Fungal Activity of Green Synthesized Copper Nanoparticles Using Plant Extract of *Bryophyllum Pinnatum* (Lam.) and *Polyalthia Longifolia* (Sonn.)

R. Hanisha, R. Udayakumar, S. Selvayogesh, P. Keerthivasan and R. Gnanasekaran*

Department of Biotechnology, Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, 60, Avadi – Vel Tech Road Vel Nagar Avadi, Chennai, Tamil Nadu, India.

http://dx.doi.org/10.13005/bbra/3091

(Received: 09 January 2023; accepted: 27 February 2023)

Plant-mediated nano-fabrication is a new area of nanotechnology that is preferred to traditional methods due to its advantages in terms of safety, cost efficiency, environmental friendliness, and biocompatibility. In the current study, copper chloride and copper sulphate are used as precursor materials to examine the effectiveness of green synthesized copper oxide nanoparticles using the plants Bryophyllum pinnatum (lam.) and Polyalthia longifolia (Sonn.) . Comparative study on the efficiency of the synthesized Copper oxide nanoparticles against each precursor has been studied. Different spectroscopic and microscopic characterization techniques such as UV- Visible spectrophotometer, X- ray powder diffraction (XRD), Fourier transformed infrared spectroscopy (FTIR), Scanning Electron Microscope (SEM) were performed to confirm the presence of copper oxide nanoparticles. UV –vis spectrophotometer results confirmed the existence of copper oxide nanoparticles using Copper chloride and Copper sulphate precursor showed absorption at 235nm and 575nm respectively. X- Ray Diffraction results showed crystalline structure of the particles with three peaks at (111), (200) & (220) which confirmed the presence of copper oxide nanoparticle for both the precursors. FTIR results supported the existence of several functional groups involved in capping, reducing, and stabilizing copper oxide nanoparticles. The SEM image showed that the copper oxide nanoparticles were spherical in shape and ranged in size from 40 to 90 nm. Further, the Anti-fungal and Anti-bacterial activity of the synthesized nanoparticle for both the copper chloride and copper sulphate precursor were studied. The Study shown maximum zone of inhibition at 100μg/ml as 18mm and 25mm respectively against Galactomyces geotrichum. As a result of the high biological potentials and powerful Antifungal activity, the green synthesized copper oxide nanoparticles can be exploited in phytopathology to combat plant infections.

Keywords: Anti- fungal activity; Anti-bacterial activity; Copper oxide nanoparticles; green synthesis; Phytopathology.

Nanoparticles can be made via chemical or biological processes. Chemical production process have been associated with a number of detrimental impacts because some dangerous chemicals are present and have been absorbed on the surface. Instead of using chemical and physical methods to create nanoparticles, biological procedures using bacteria, enzymes, fungus, plants, or plant

*Corresponding author E-mail: gnanasekaranbio@gmail.com

extracts are a suitable alternative[1]. One important area of nanotechnology is the creation of these environmentally benign processes for generating nanoparticles, especially copper nanoparticles, which have various applications[2].

Some microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* microscopic organisms, are poisoned by copper nanoparticles, but animal cells are unaffected. Using the "green mix" method, certain odd plants and nanoparticles have been combined. A few odd plants and nanoparticles have been combined using the green union technique[3]. Copper nanoparticles are responsive and can surely work with other particles due to their significant surface area to volume ratio. For a very long time, colloidal copper has been used as a potent antimicrobial. Because of the use of copper nanoparticles in wound dressings and their biocidal properties, scientists have given them a lot of thought. [4]Virulent species like *Staphylococcus aureus*, are susceptible to the anticipated antibacterial actions of copper nanoparticles. Due to their extensive uses in fields like mechanics, optics, biomedical sciences, hardware, sedate quality conveyance, catalytic photo electrochemical applications, and nonlinear optical devices, these nanoparticles have also caught the attention of experts.[3,5]

Bryophyllum pinnatum (lam.)(BP) (equivalent word: Kalanchoepinnata) is a perpetual spice developed and utilized in folkloric medication in tropical Africa, India, China, Australia and tropical America[6] Figure 1. In a creating nation like Nigeria, BP is delegated a weed that thrives all through the Southern piece of the nation. These plants are typically found in gardens wealthy in natural fertilizer and adequate dampness.[7] Various dynamic mixes, including flavonoids, glycosides, steroids, bufadienolides and natural acids, have been distinguished in *Bryophyllum pinnatum*.[8] In this manner, BP is utilized for medication in ethno practice treatment of injuries of the navel of recently conceived children, in ear problem, hack, looseness of the bowels, and diarrhea. It was discovered that the leaves 60% methanolic extract was efficient against several bacteria. These include *Staphylococcus aureus, Shigella dysenteriae, Proteus vulgaris, Bacillus substilis, and Escherichia coli* at a dosage of 25 mg/ml. The extract was found to be ineffective against *Klebsiella pneumonia, Candida albicans, and Pseudomonas aeruginosa*[9].

Polyalthia longifolia (Sonn.) the false ashoka native to India, is an elevated evergreen tree, normally planted because of its viability in all conditions. A massive variety of bushes and trees known as *Polyalthia longifolia (Sonn.)* is found across the tropics and subtropics[10] Figure 2. It belongs to the Annonaceae family, which has more than 2000 species and 120 genera. *Polyalthia longifolia (Sonn.)* Pendular is a tall, beautiful, evergreen tree native to Sri Lanka that has spread over the Indo-Pakistan sub-mainland. It has a straight trunk and flat branches[10]. The bark of *Polyalthia longifolia (Sonn.)* is cited in ethno pharmacological claims to have febrifuge properties. It weakens the heart, lessens circulatory strain, and energizes breathing. It has been taken into account how *Polyalthia longifolia (Sonn.)* affects fungus. It is useful in treating helminthiasis, diabetes, hypertension, and skin conditions. Additionally, it is applied to the healing of wounds.[11]

Methanol extracts of *Polyalthia longifolia (Sonn.)* var. pendula's leaves, stems, twigs, green berries, flowers, roots, root-wood, and rootbark were tested for their antimicrobial and antifungal properties.[12] With MIC values between 7.8 and 500 g/ml, the methanol extract of leaves and berries completed bioassay-monitored isolation operations and showed good antibacterial activity.[13] 91 clinically relevant microbial species were tested for their antibacterial potential by two dosages of various *P. longifolia* leaf extracts, including 1, 4-dioxan, methanol, and acetone extracts.[14] All three plant extracts are efficient against 98% of the total number of gram-positive bacterial strains at a concentration of 500 g/disc.[15] While 1, 4-dioxan extract was efficacious against 18.18% of the total gram negative bacterial strains, methanolic and acetone extracts were only effective against 12.72 % of them.[16]

Copper has a wide scope of antimicrobial activity including bacterial species and is viewed as more powerful than costly metallic antimicrobials.[17] Copper kills microorganisms by an instrument called contact executing. Copper Nanoparticles can be used as microbial development inhibitors.[18] Additionally, metallic nanoparticles have a biocidal effect on both bacterial species. They are bactericidal because of their high surface to volume ratio and small size.[19] They become highly intensive as a result, disrupting microbial membranes. The aqueous extract of two plant leaves and copper sulphate pentahydrate were used in this study to create copper nanoparticles (CuNP), which were subsequently analyzed utilizing cutting-edge techniques.[20] Bacterial and fungal species were examined for their susceptibility to the produced CuNP antimicrobial properties.[21]

MATERIALS AND METHODS

Preparation to Plant extract

Bryophyllum pinnatum (lam.) and *Polyalthia longifolia (Sonn.)* leaves were purchased from nursey nearby Avadi. The collected leaves were washed with running water then by distilled water and dried in shade for not more than 10 days. The shade dried leaves were gathered and squashed using mortar and chisel, followed which the leaves were taken in a receptive and 100ml of refined water is added and heated in water bath for 100ÚC for 30 minutes Figure 3a and 3b. Then the extract was collected using Whatman No.1 filter paper and used in the synthesis of nanoparticles.[22,23]

Green Synthesis of Copper nanoparticles

Copper nanoparticles were produced by 1ml of plant extract in to 30ml of copper chloride and copper sulphate solution while stirring for 30 minutes. The two different preparations were stored at darkroom overnight by wrapping in aluminum foil Figure 4a and 4b. The copper solutions original blue tint changed to an earthy green hue, visually confirming the preliminary method's assertion that nanoparticles had formed. Both the solution were centrifuges at 10000 rpm after the change of color. After discarding the supernatant, the formatted Copper nanoparticles Figure 5a and 5b were washed with distilled water and characterization analysis is done. [24]

Characteristics analysis

Shimadzu's UV-2600 UV-visible spectrophotometer was used to capture the samples' UV-visible absorbance and reflectance spectra. Its wavelength range is 200-800 nm. UV-DRS diffuse reflection spectroscopy, whereas transmission was utilized to analyze UV-visible spectra. The plant extract was combined with copper solution after the necessary dilution to create the Copper nanoparticle solution. An absorbance spectrum was discovered by contrasting the absorbance of a nanoparticle solution with that of a control extract solution at wavelengths ranging from 200 to 800 nm. FTIR analysis for leaf extracts of *Polyalthia longifolia (Sonn.)* and *Bryophyllum pinnatum (lam.)* using copper sulphate and chloride, respectively, as precursors. The terms "FTIR analysis" and "FTIR spectroscopy" are frequently used to describe Fourier transform infrared spectroscopy. The analytical technique known as FTIR analysis can be used to identify organic, polymeric, and occasionally inorganic functional groups. This method uses infrared light to scan test samples and look at chemical properties [25].

RESULTS AND DISCUSSION

Characterization of nanoparticle

The synthesized copper oxide nanoparticles optical characteristics along with the biocomponents found in plants. Crystallinity, nanoparticle size, and shape were all examined throughout their production utilizing a variety of techniques[26]. The produced nanoparticles were examined using FTIR (Fourier Transform Infrared Spectroscopy), UV-Visible spectroscopy, XRD analysis, and SEM examination[27].

Fourier Transform Infrared Spectroscopy

A FTIR Spectrometer analysis was performed to determine the likely functional groups in plant extract that are involved in the synthesis, stabilization of copper oxide nanoparticles. The spectra between 650 and 4000 cm^{-1} were acquired FT-IR characterization used to examine the phytocomponents from plants that are in converting cupric ions to copper oxide nanoparticles and then capping them. The peaks of the spectrum of *Bryophyllum pinnatum (lam.)* plant extract include 2355cm^{-1}, 3312cm^{-1}, 1640cm^{-1}, 3350cm^{-1}, 2322cm^{-1}, 3327cm^{-1}, and 776cm^{-1}. FTIR examination revealed the hydroxyl (-OH) group with a peak at 3312cm^{-1}, whereas the N-H peak is at 3350cm^{-1}. The peak at 2,355 cm^{-1} was attributed to CN, while the peak at 3312 cm^{-1} belongs to CC-H. The peak C=O stretch measurement is 1640 cm^{-1}. In the FTIR spectrum of copper oxide nanoparticles, the peculiar characteristic bands at 773, 974, 1074, 1097, 1119, 1153, 1339, 1383, 1633, and 3231cm^{-1} correspond to the bands from the plant extract of *Bryophyllum pinnatum*. The N-H extension vibration is attributed to the peak at 3231cm^{-1}.1633 C=N by 1383, C=C by 1339, C=O by 1153, and C=O by 1119 Figure 6. This suggests that copper oxide nanoparticles were produced using biomolecules that can be found in plant extract[28].

The phytochemicals from plants that are engaged in reducing copper ions to copper oxide nanoparticles and afterwards capping were also analyzed using FT-IR characterization. The peaks in the spectrum of *Polyalthia longifolia (Sonn.)* leaf extract include which was evidently seen at 3354, 2922, and 1744 cm^{-1}, respectively, and corresponds to the O-H, C-H, and C = O stretching vibrations of polyphenol compounds. Simultaneously, the interaction between copper-based nanoparticles and polyphenols results in a broad absorption peak at 3356 and 2926 cm^{-1} of O-H and C-H stretching. O-H stretching was substantially less intense as a result of the Cu ion's oxidation process, which produced the Cu(II), Cu(I), and Cu(0) ions. The peak intensity at 1635 cm^{-1} was therefore attributed to the C = O stretching from the polyphenol bond with Cu metal, leading to a decreased electron density as a result of the dispersion of electrons towards Cu ions Figure 7. The extract also functions as a stabilizing agent (which prevents re-oxidation) in addition to acting as a reducing agent[29].

XRD Analysis

Figure 8 displays the XRD result of copper oxide nanoparticles produced using *Bryophyllum pinnatum (lam.)* plant extract. 2 Diffraction peaks were found at 11.763°, 12.838°, 20.782°, 25.777°, 42°, and 57.06°. Strong peaks in XRD patterns indicate that the CuO nanoparticles produced by reducing Cu ions in the presence of *Bryophyllum pinnatum (lam.)* leaf extracts are crystalline. The JCPDS/ICDD database and published literatures were compared to the miller indices and peaks of

Fig. 1. Leaf of *Bryophyllum pinnatum (lam.)*

Fig. 2. Leaf of *Polyalthia longifolia (Sonn.)*

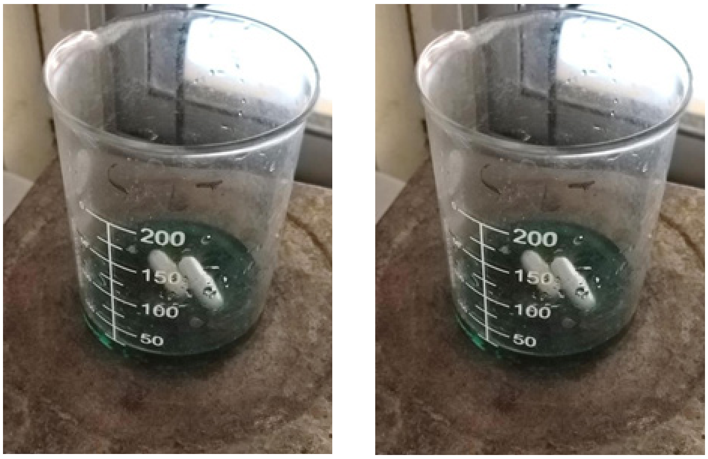

Fig. 3a. Leaf extract of *Bryophyllum pinnatum (lam.)* with $CuCl_2$ as precursor **3b.** Leaf extract of *Polyalthia longifolia (Sonn.)* with $CuSo_4$ as precursor.

CuO nanoparticles using the Jade software, and it was discovered that they were fairly close. The copper oxide crystal phase's (113), (030), (036), and (436) planes are linked to the diffraction peaks at 2 values of 20.78°, 25.77°, 42°, and 57.06°, respectively. Although cuprous oxide (CuO crystalline's) phase peaks at 11.763° and 12.83° are assigned to its (110), (110), and (220) planes, respectively, the results are in very good agreement with powder CuO from the JCPDS standards (JCPDS file no. 77-1898). These values match reported literature and the values for the cubic phase of CuO match the corresponding "JCPDS" (Joint Committee on Powder Diffraction Standards card no. 34-1354).[30] Both oxides are present in the crystalline phase of CuO nanoparticles, as shown by the presence of CuO and CuO-related peaks in the XRD spectrum. The average crystal size of CuONPs is 65.776 nm. XRD analysis has been used in a few publications to describe different crystalline sizes for CuONPs that were bio-produced utilizing plant extracts.[31]

The considerable absorption of the plant extract, which was clearly detected at 3354, 2922, and 1744 cm^{-1}, respectively, corresponds to the O-H, C-H, and C = O stretching vibrations of the polyphenolic compound. Figure 9 illustrates this. A large absorption peak of O-H and C-H stretching is produced at 3356 and 2926 cm^{-1} by the interaction of polyphenol and Cu-based NPs. O-H stretching

Fig. 4a. Leaf extract of *Bryohyllum pinnatum (lam.)* with $CuCl_2$ as precursor **4b**. Leaf extract of *Polyalthia longifolia (Sonn.)* with $CuSo_4$ as precursor at zero hours

Fig. 5a. Leaf extract of *Bryohyllum pinnatum (lam.)* with $CuCl_2$ as precursor **5b.** Leaf extract of *Polyalthia longifolia (Sonn.)* with $CuSo_4$ as precursor at 24 hours

was substantially less intense as a result of the Cu ion's oxidation process, which produced the Cu(II), Cu(I), and Cu(0) ions. In order to explain the peak intensity at 1635 cm^{-1}, it was determined that the C = O stretching from the polyphenol bond with Cu metal caused a drop in electron density as a result of the reoxidation process (stabilizing agent). The polyphenol compound's O-H bond to copper ions

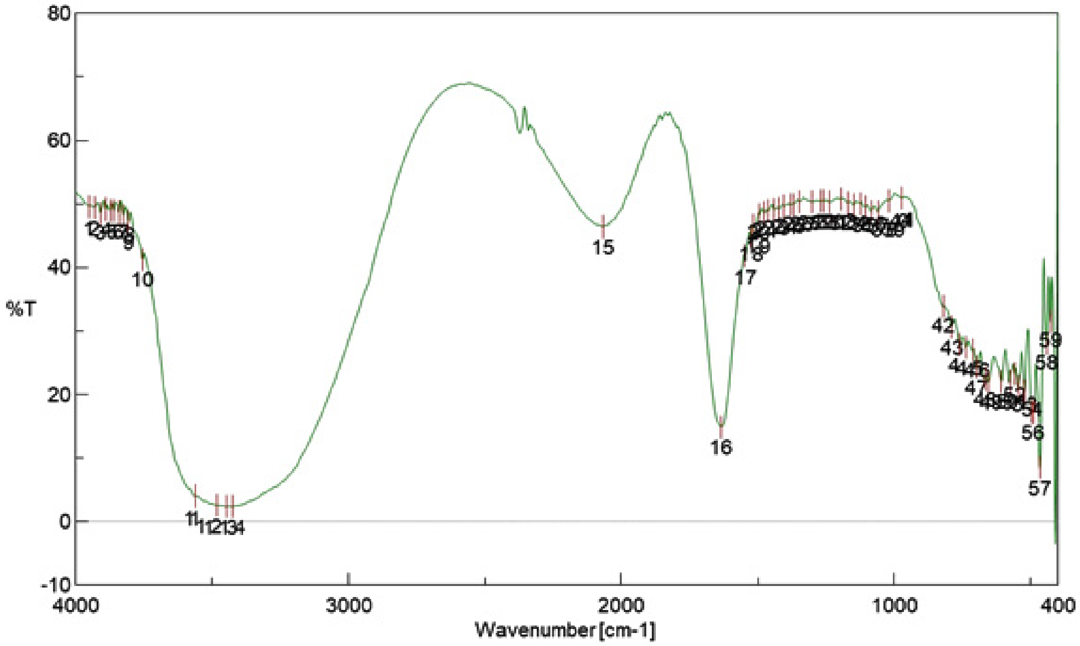

Fig. 6. FTIR Result of green synthesized CuONP with leaf extract and $CuCl_2$ as precursor

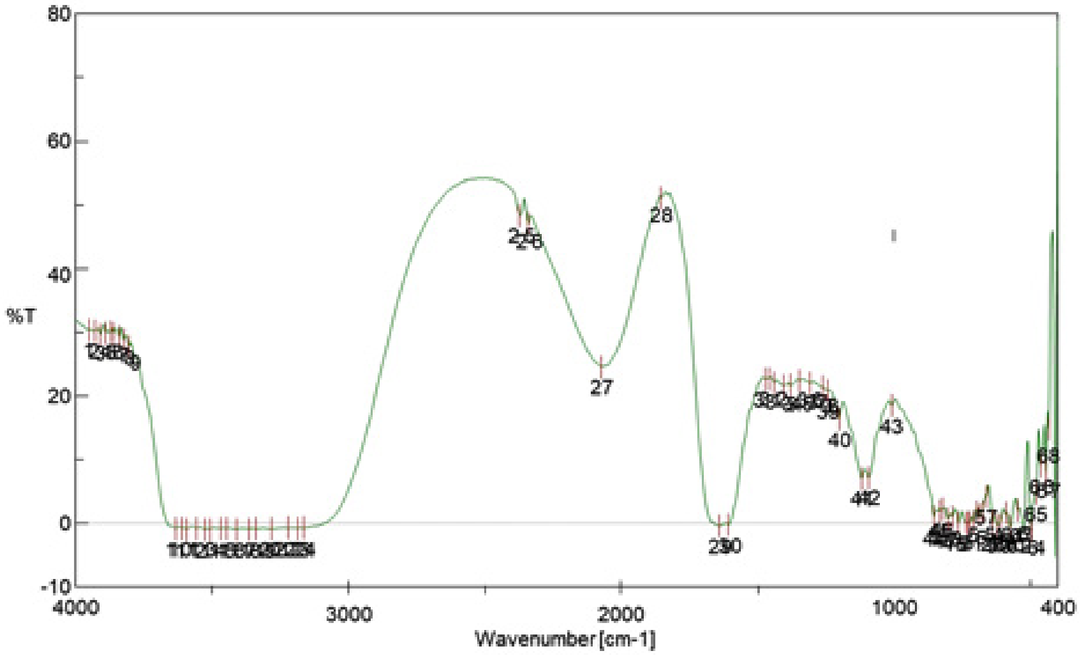

Fig. 7. FTIR Result of green synthesized CuONP with leaf extract and $CuSO_4$ as precursor

was further confirmed by the absorption at 1095 cm^{-1} [32]

UV-Visible Spectrophotometer

An illustration of the creation of copper oxide nanoparticles is the change in colour of the prepared formulation from blue to green completing the reactino between extract of the plant and copper ions. This result mirrors what was discovered in earlier research[33,34]. Additionally, the absorbance was looked at, and it has been

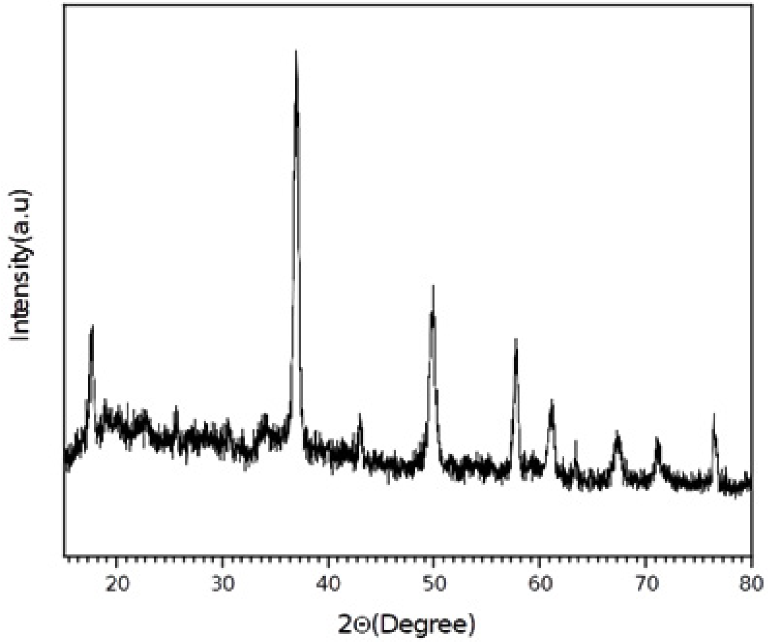

Fig. 8. FTIR Result of green synthesized CuONPs with leaf extract and $CuCl_2$ as precursor

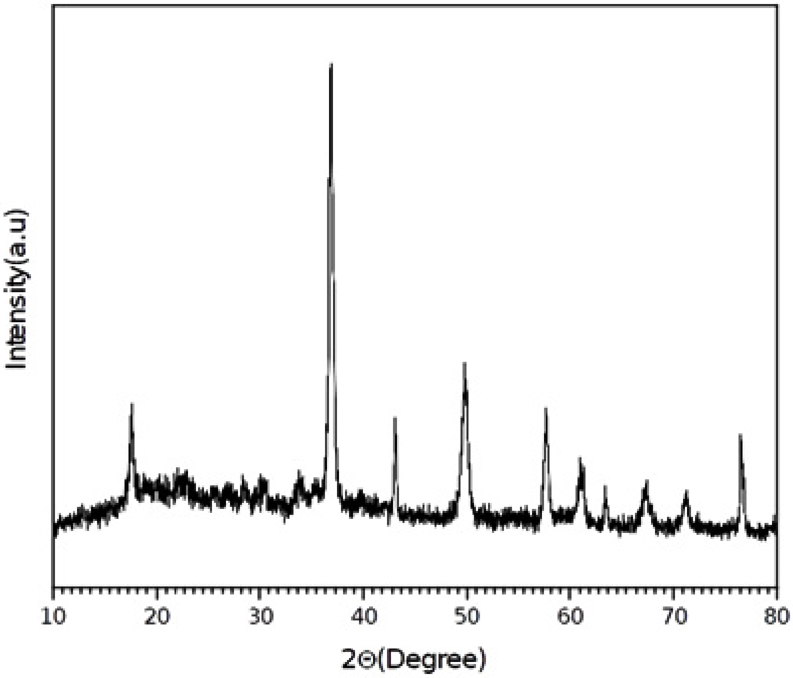

Fig. 9. FTIR Result of green synthesized CuONPs with leaf extract and $CuSO_4$ as precursor

discovered that the highest value of copper oxide nanoparticles was seen at 434 nm Figure 10, which is consistent with earlier research. At wavelengths of 434 nm, the absorption peaks of copper oxide nanoparticles produced by *Bryophyllum pinnatum (lam.)* where terpenoids act as a capping agent were visible,[35] while copper oxide nanoparticles made from *Polyalthia longifolia (Sonn.)* had peak at 730nm[36] Figure 11. The production of copper oxide nanoparticles is readily visible in the UV-vis spectrum between 450 and 700 nm via plasmon resonance. [37]

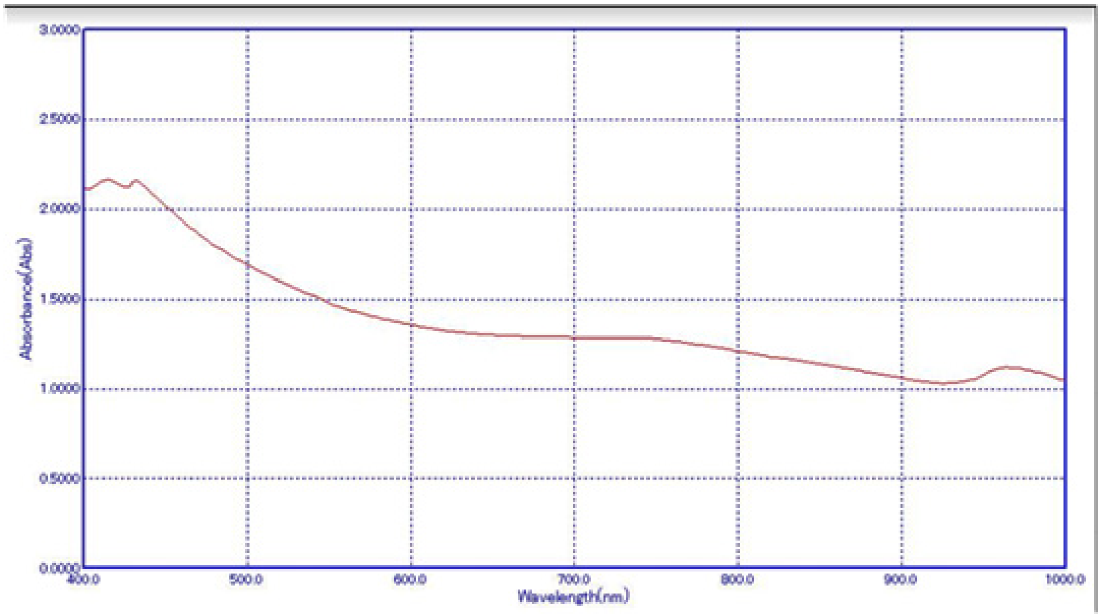

Fig. 10. UV Result of green synthesized CuONPs with leaf extract and $CuCl_2$ as precursor

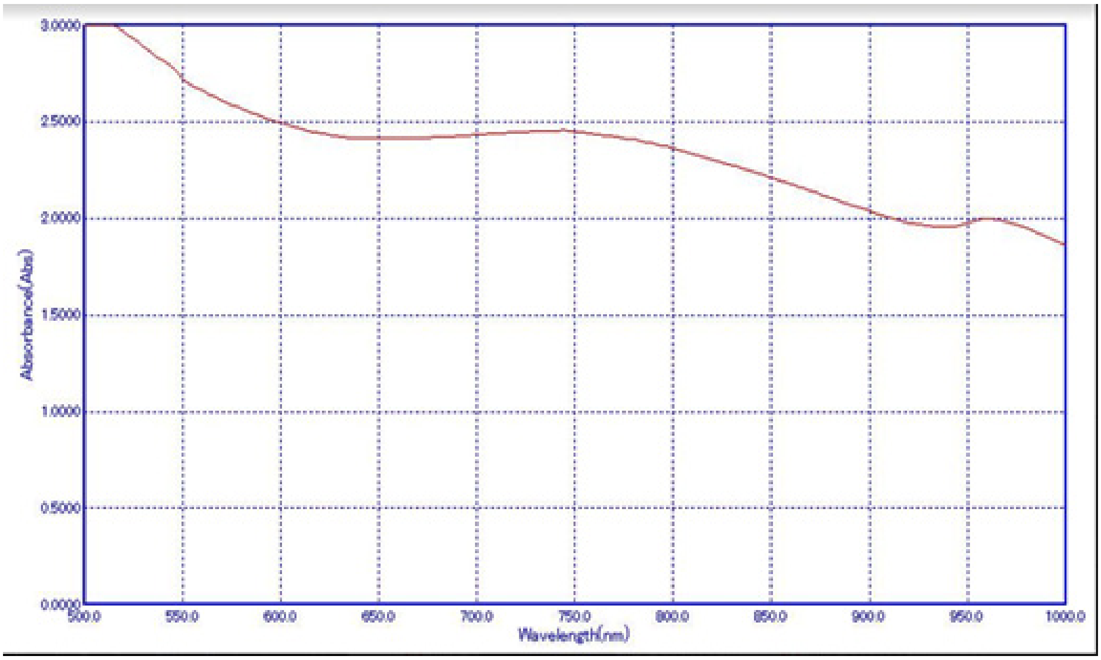

Fig. 11. UV Result of green synthesized CuONPs with leaf extract and $CuSO_4$ as precursor

SEM Analysis

Utilizing HR FESEM images, the morphological properties of the produced Copper nanoparticles using *Bryophyllum pinnatum (lam.)* and *Polyalthia longifolia (Sonn.)* were identified. The produced Copper oxide nanoparticles SEM pictures showed that they are crystalline in structure and range in size from 78 to 134 nm.

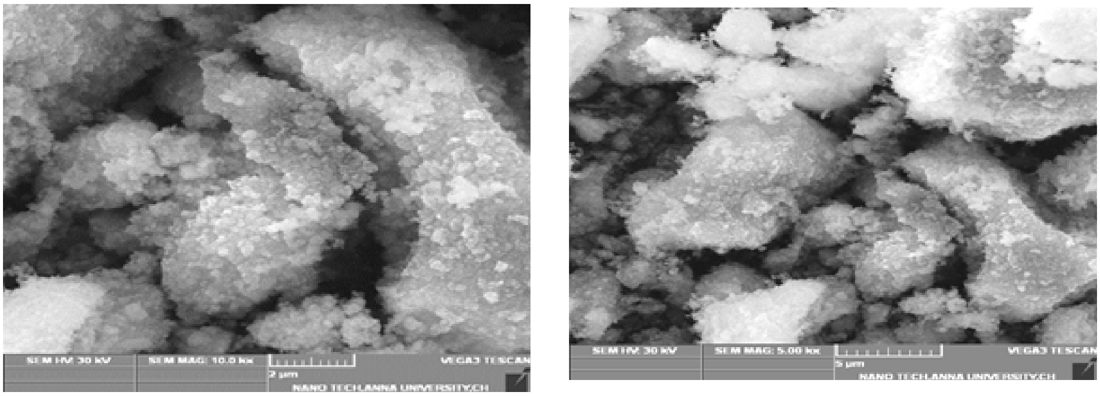

Fig. 12. SEM result of green synthesized CuO NPs with leaf extract and $CuCl_2$ as precursor

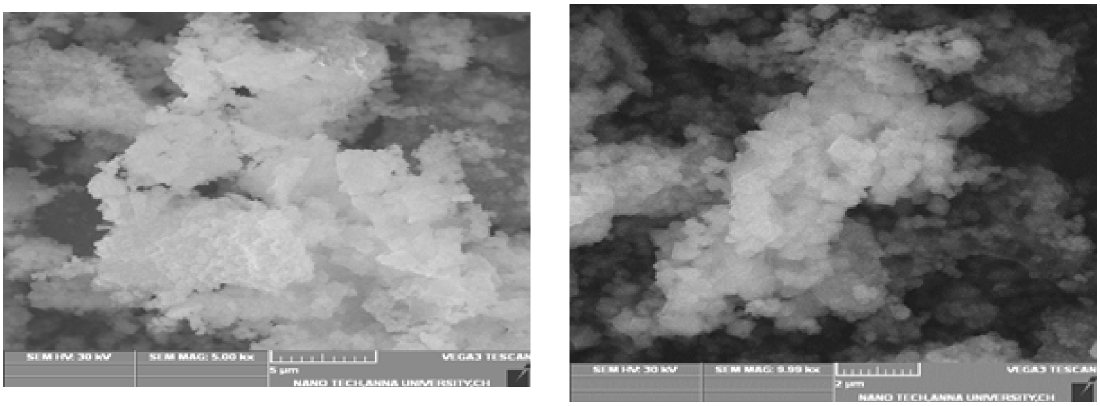

Fig. 13. SEM result of green synthesized CuO NPs with leaf extract and $CuSo_4$ as precursor

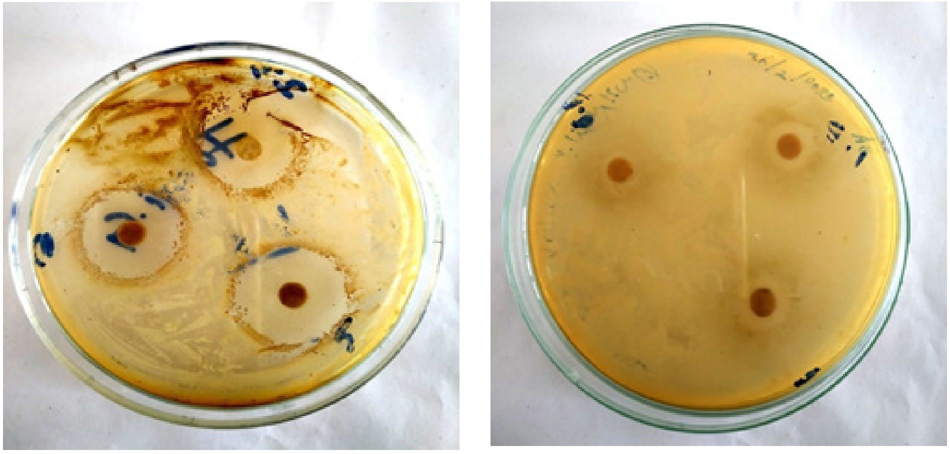

Fig. 14. Antifungal activity of copper oxide nanoparticle with $CuCl_2$ as precursor

Anti-fungal activity of copper oxide nanoparticle

An agar plate method was used to examine the antifungal activity of the copper oxide nanoparticles against *Galactomyces geotrichum*. After 24 hours of incubation with copper oxide nanoparticles, an 18mm and 25mm figure 14 zone of inhibition was discovered using the agar plate method, respectively. According to the outcome, a higher inhibition zone was observed. Greater inhibition zones in the agar plate test have been observed in several studies with comparable results[38 39].

CONCLUSIONS

Copper nanoparticles were successfully synthesized by green method, in which copper sulphate and copper chloride were used as a precursor. The green reduction of copper ions by flavonoids such as Kaempferol, terpenoids and organic acids involves change of color from copper blue to dark brown after 24 hours which preliminarily confirms the presence of copper nanoparticles. The Characterization analysis by UV-Vis Spectrophotometer, FTIR, XRD and SEM confirmed the presence of copper nanoparticles in the size of 120-150nm. The green synthesized copper nanoparticles exhibited high anti- fungal activity against *Galactomyces geotrichum* with the inhibition zone of 14mm for copper chloride as a precursor and 25mm for copper sulphate as a precursor. This work proposes a facile and cheap green synthesis method for the production of copper nanoparticles with high antifungal activity. Thus, the nanomaterial could be useful for controlling pathogenic fungi for various applications.

ACKNOWLEDGMENT

Authors are thankful to the management of Vel tech High tech Rangarajan Dr. Sakunthala Engineering College, Chennai for their constant support to complete this research paper.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding Sources

The article was entirely financed by the author themselves.

REFERENCES

1. Farré M, Gajda-Schrantz K, Kantiani L, Barceló D. Ecotoxicity and analysis of nanomaterials in the aquatic environment. *Anal Bioanal Chem*. 2009;393(1):81-95.
2. Parveen K, Banse V, Ledwani L. Green synthesis of nanoparticles: their advantages and disadvantages. In: *AIP Conference Proceedings*. Vol 1724. ; 2016:20048.
3. Shende S, Ingle AP, Gade A, Rai M. Green synthesis of copper nanoparticles by Citrus medica Linn.(Idilimbu) juice and its antimicrobial activity. *World J Microbiol Biotechnol*. 2015;31(6):865-873.
4. Tamayo L, Azócar M, Kogan M, Riveros A, Páez M. Copper-polymer nanocomposites: An excellent and cost-effective biocide for use on antibacterial surfaces. *Materials Science and Engineering: C*. 2016;69:1391-1409.
5. Ortiz-Benitez EA, Carrillo-Morales M, Velázquez-Guadarrama N, Fandiño-Armas J, Olivares-Trejo J de J. Inclusion bodies and pH lowering: as an effect of gold nanoparticles in Streptococcus pneumoniae. *Metallomics*. 2015;7(7):1173-1179.
6. Singh S, Verma P, Verma P, et al. A Review on In Vitro Culture Method and Assessment of Therapeutic potential of *Bryophyllum pinnatum (lam.)*(Lam.).
7. Mayekiso A. *Economic Assessment of Indigenous Leafy Vegetables (ILVs) Production for Income Generation and Food Income Generation and Food Security in the Eastern Cape Province, South Africa*. 2021.
8. Alyamani AA, Albukhaty S, Aloufi S, AlMalki FA, Al-Karagoly H, Sulaiman GM. Green Fabrication of Zinc Oxide Nanoparticles Using Phlomis Leaf Extract: Characterization and In Vitro Evaluation of Cytotoxicity and Antibacterial Properties. *Molecules*. 2021;26(20):6140. doi:10.3390/molecules26206140
9. Saeidi S, Hassanpour K, Ghamgosha M, et al. Antibacterial activity of ethyl acetate and aqueous extracts of Mentha longifolia L. and hydroalcoholic extract of Zataria multiflora Boiss. plants against important human pathogens. *Asian Pac J Trop Med*. 2014;7:S186—S189.
10. Perry DA, Oren R, Hart SC. *Forest Ecosystems*. JHU press; 2008.
11. Singh R, Upadhyay SK, Rani A, et al. Ethanobotanical study of Subhartipuram, Meerut, Uttar Pradesh, India. I. Diversity and pharmacological significance of trees. *International Journal of Pharmaceutical Research*. 2019;11(4):782-794.

12. Katkar K V, Suthar AC, Chauhan VS. The chemistry, pharmacologic, and therapeutic applications of Polyalthia longifolia (Sonn.) . *Pharmacogn Rev*. 2010;4(7):62.
13. Nguyen HT, Yu NH, Park AR, Park HW, Kim IS, Kim JC. Antibacterial activity of pharbitin, isolated from the seeds of Pharbitis nil, against various plant pathogenic bacteria. *J Microbiol Biotechnol*. 2017;27(10):1763-1772.
14. Kekuda TRP, Dileep N, Rakesh KN, Syed J, Raghavendra HL. Elemental analysis and bioactivities of ripe and unripe pericarp of *Polyalthia longifolia (Sonn.)* (Annonaceae). *Science, Technology and Arts Research Journal*. 2014;3(2):68-75.
15. Vijayakumar A, Duraipandiyan V, Jeyaraj B, Agastian P, Raj MK, Ignacimuthu S. Phytochemical analysis and in vitro antimicrobial activity of Illicium griffithii Hook. f. \& Thoms extracts. *Asian Pac J Trop Dis*. 2012;2(3):190-199.
16. Lavanya C, Rao BG, Ramadevi D. Phytochemical and pharmacological studies on Polyalthia longifolia (Sonn.) . *Pharm Sci Res*. 2018;3(4):1-7.
17. Ogunsona EO, Muthuraj R, Ojogbo E, Valerio O, Mekonnen TH. Engineered nanomaterials for antimicrobial applications: A review. *Appl Mater Today*. 2020;18:100473.
18. Castresana PA, Martinez SM, Freeman E, Eslava S, Di Lorenzo M. Electricity generation from moss with light-driven microbial fuel cells. *Electrochim Acta*. 2019;298:934-942.
19. Dadi R, Azouani R, Traore M, Mielcarek C, Kanaev A. Antibacterial activity of ZnO and CuO nanoparticles against gram positive and gram negative strains. *Materials Science and Engineering: C*. 2019;104:109968.
20. Ahmadi-Tehrani D. *Polymeric, Wurster-Type Hosts For The Encapsulation Of Metal Cations And Molecular Guests*. 2020.
21. Mai-Prochnow A, Clauson M, Hong J, Murphy AB. Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. *Sci Rep*. 2016;6(1):1-11.
22. Fentie M, Chouhan G, Moges M, Tyagi P. Green synthesis of copper oxide nanoparticles using *Bryophyllum pinnatum (lam.)*leaf extract and its antibacterial potential against Listeria monocytogenes. *Int J Health Sci (Qassim)*. (II):5349-5367.
23. Alhujaily M, Albukhaty S, Yusuf M, et al. Recent Advances in Plant-Mediated Zinc Oxide Nanoparticles with Their Significant Biomedical Properties. *Bioengineering*. 2022;9(10):541. doi:10.3390/bioengineering9100541
24. Din MI, Rehan R. Synthesis, characterization, and applications of copper nanoparticles. *Anal Lett*. 2017;50(1):50-62.
25. Kadhim AA, Abbas NR, Kadhum HH, et al. Investigating the Effects of Biogenic Zinc Oxide Nanoparticles Produced Using Papaver somniferum Extract on Oxidative Stress, Cytotoxicity, and the Induction of Apoptosis in the THP-1 Cell Line. *Biol Trace Elem Res*. Published online January 20, 2023. doi:10.1007/s12011-023-03574-7
26. Jihad MA, Noori FTM, Jabir MS, Albukhaty S, AlMalki FA, Alyamani AA. Polyethylene Glycol Functionalized Graphene Oxide Nanoparticles Loaded with Nigella sativa Extract: A Smart Antibacterial Therapeutic Drug Delivery System. *Molecules*. 2021;26(11):3067. doi:10.3390/molecules26113067
27. Kumar A, Dixit CK. Methods for characterization of nanoparticles. In: *Advances in Nanomedicine for the Delivery of Therapeutic Nucleic Acids*. Elsevier; 2017:43-58.
28. Mehdizadeh T, Zamani A, Froushani SMA. Preparation of Cu nanoparticles fixed on cellulosic walnut shell material and investigation of its antibacterial, antioxidant and anticancer effects. *Heliyon*. 2020;6(3):e03528.
29. Maulana I, Fasya D, Ginting B. Biosynthesis of Cu nanoparticles using *Polyalthia longifolia (Sonn.)* roots extracts for antibacterial, antioxidant and cytotoxicity applications. *Materials Technology*. Published online 2022:1-5.
30. Nainwal LM, Tasneem S, Akhtar W, et al. Green recipes to quinoline: A review. *Eur J Med Chem*. 2019;164:121-170.
31. Khan A, Rashid A, Younas R, Chong R. A chemical reduction approach to the synthesis of copper nanoparticles. *Int Nano Lett*. 2016;6(1):21-26.
32. Bisht S, Sharma V, Kumari N. Biosynthesized magnetite nanoparticles from *Polyalthia longifolia (Sonn.)* leaves improve photosynthetic performance and yield of Trigonella foenum-graecum under drought stress. *Plant Stress*. 2022;5:100090.
33. Gebremedhn K, Kahsay MH, Aklilu M. Green synthesis of CuO nanoparticles using leaf extract of catha edulis and its antibacterial activity. *Journal of Pharmacy and Pharmacology*. 2019;7(6):327-342.
34. Sepasgozar SME, Mohseni S, Feizyzadeh B, Morsali A. Green synthesis of zinc oxide and copper oxide nanoparticles using Achillea Nobilis extract and evaluating their antioxidant and antibacterial properties. *Bulletin of Materials Science*. 2021;44(2):1-13.

35. Sagadevan A, Greaney MF. meta-Selective C- H activation of arenes at room temperature using visible light: dual-function ruthenium catalysis. *Angewandte Chemie International Edition*. 2019;58(29):9826-9830.
36. Chaudhary S, Rohilla D, Umar A, Kaur N, Shanavas A. Synthesis and characterizations of luminescent copper oxide nanoparticles: toxicological profiling and sensing applications. *Ceram Int*. 2019;45(12):15025-15035.
37. Sankar R, Manikandan P, Malarvizhi V, Fathima T, Shivashangari KS, Ravikumar V. Green synthesis of colloidal copper oxide nanoparticles using Carica papaya and its application in photocatalytic dye degradation. *Spectrochim Acta A Mol Biomol Spectrosc*. 2014;121:746-750.
38. Gunalan S, Sivaraj R, Rajendran V. Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Progress in Natural Science: Materials International*. 2012;22(6):693-700.
39. Hamelian M, Hemmati S, Varmira K, Veisi H. Green synthesis, antibacterial, antioxidant and cytotoxic effect of gold nanoparticles using Pistacia Atlantica extract. *J Taiwan Inst Chem Eng*. 2018;93:21-30.