ISSN 1996-5044

Research Journal of

Nanoscience and Nanotechnology

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS Research Journal of Nanoscience and Nanotechnology

ISSN 1996-5044
DOI: 10.3923/rjnn.2024.1.7

Research Article
Biosynthesis of Copper Oxide Nanoparticles Formation from *Pongamia pinnata* (L.) Pierre Leaf Metabolites and Their Antibacterial Activity

[1]Parsotam Tramta, [2]Kalpna Rakholiya and [1]Mital Kaneria

[1]Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat 360005, India
[2]Department of Microbiology, Harivandana College, Rajkot, Gujarat 360005, India

Abstract

Background and Objective: Bacterial resistance is one of the biggest challenges in the world. In this point of view, the present study was designed to synthesize copper nanoparticles using leaf aqueous extract of *Pongamia pinnata* in search of a new source to overcome the issue of drug resistance. **Materials and Methods:** Copper Oxide Nanoparticles (CuO NPs) were synthesized using *Pongamia pinnata* leaf extract. The plant extract was prepared using the decoction extraction method. The characterization was done by various spectral analyses namely UV-Vis spectroscopy, FTIR analysis, SEM and Zeta potential analysis. The antibacterial effect was evaluated by the agar disc diffusion method. **Results:** The synthesis of CuO NPs was confirmed by its characteristic peak at 339 nm by UV-Vis spectra, while SEM revealed the size in the range of 40.3-101.2 nm and the average size was 58.1 nm. The FTIR confirmed functional groups like alcohol, amines, alkyne, nitrile and halogen in *P. pinnata* leaf extract. The biosynthesized ZuO NPs showed potent antibacterial activity against four microorganisms, viz *Staphylococcus aureus* ATCC25923, *Listeria monocytogenes* ATCC19112, *Salmonella typhimurium* ATCC23564 and *Escherichia coli* NCIM2931. **Conclusion:** The green synthesized *P. pinnata* leaf-mediated CuO NPs can be further explored as a potential candidate for the antibacterial agent.

Key words: Nanotechnology, copper oxide nanoparticles, *Pongamia pinnata*, green synthesis, antibacterial, leaf

Citation: Tramta, P., K. Rakholiya and M. Kaneria, 2024. Biosynthesis of copper oxide nanoparticles formation from *Pongamia pinnata* (L.) pierre leaf metabolites and their antibacterial activity. Res. J. Nanosci. Nanotechnol., 14: 1-7.

Corresponding Author: Mital Kaneria, Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat 360005, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nanoparticles have gained enormous significance due to their broad range of applications in numerous areas like biotechnology, biophysics, biomedical, biochemistry, pharmaceutical science, bioengineering, etc. Nanoparticles are synthesized from different metals like silver, gold, titanium, platinum, zinc, magnesium and copper. Their distinctive size-dependent properties make them superior and very applicable[1]. Plant extract-mediated synthesis is more advantageous than other methods due to the rapid reduction of metal ions and more stable nanoparticles are formed. The green synthesis is simple, easy, cost-effective, rapid and eco-friendly[2]. Plants have numerous phytochemicals like phenols, alkaloids, steroids, tannins, flavonoids, saponins, etc., responsible for the reduction and stabilization of nanoparticles[3].

Copper nanoparticles synthesized using different plant parts are *Millettia pinnata* flower[4], *Aquilaria malaccensis* leaf[5], *Fragaria ananassa* leaf[6], etc. The CuO NPs also possess various biological activities like anticancer[7], antimutagenicity[8], antifungal[9], antioxidant[10], larvicidal[11], antimicrobial[12], antidiabetic[4], etc.

Pongamia pinnata (Family: Fabaceae) is found throughout India. The medicinal properties of *P. pinnata* are well described in the literature as well as plant is also reported for the presence of a number of secondary metabolites and oil[13]. Roots, bark, leaves, flowers and seeds of this plant also have medicinal properties and are traditionally used. *Pongamia pinnata* is known to possess various pharmacological activities like anti-inflammatory[14], antihyperglycemic[15], analgesic[16], anticonvulsant[17], antidiabetic[18], antioxidant[19], etc. Considering the aforesaid, in the present work an attempt was made to synthesize CuO NPs using *Pongamia pinnata* leaf extract.

MATERIALS AND METHODS

Study area: The present study was conducted from June, 2022 to April, 2023 at the Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India.

Plant materials: The fresh leaves of *Pongamia pinnata* were collected from Rajkot, Gujarat, India.

Preparation of the plant extract: The plant extract for the synthesis of CuO NPs was prepared by the decoction extraction method[20]. Leaves were cleaned using deionized water. Five grams of plant materials were extracted using the decoction method for the synthesis of CuO NPs.

Synthesis of copper oxide nanoparticles: Aqueous solution of $CuSO_4$ (1 mM) was prepared and used for the synthesis of CuO NPs. As 30 mL of leaf extract was added to 170 mL of 1 mM $CuSO_4$ solution for the reduction of Cu^{2+} ions. The synthesis of copper nanoparticles was carried out at room temperature (25 ± 2°C) for 24 hrs in the dark. The copper nanoparticle solution was purified by repeated centrifugation at 10,000 rpm for 15 min followed by dispersion of the pellet of CuO NPs into acetone. After air drying, the purified copper particles were stored at 4°C for further analysis.

Characterization of the synthesized CuO Nps

UV-Vis spectroscopy: The reduction of the Cu^{2+} ions was monitored by measuring the UV-Vis spectrum of the reaction medium at different time intervals. The UV-Vis spectroscopic analysis with range of 200-800 nm was monitored as a function of time of reaction on a spectrophotometer (Shimadzu UV -1800).

Fourier-Transform Infrared Spectroscopy (FTIR) analysis: Probable functional groups associated in the synthesis and stabilization of CuO NPs were studied by FTIR spectroscopy. The FTIR spectra of leaves synthesized CuO NPs were recorded in the range of 400-4000 cm^{-1} Nicolet IS10 (Thermo Scientific, USA) The various modes of vibrations were identified and assigned to determine the presence of different functional groups.

Scanning electron microscopy (SEM): The SEM analysis was done using the Hitachi S-4500 SEM machine. A thin film of the sample was prepared on a carbon-coated copper grid, the film on an SEM grid was allowed to dry putting it under a mercury lamp.

Zeta potential analysis: Zeta potential is an essential parameter for the determination of the stability of nanoparticles. The zeta potential measurement was performed using a Microtra (Zetatra Instruments).

Antibacterial activity: The antibacterial activity CuO NPs was determined against two Gram-positive bacteria (*Staphylococcus aureus* ATCC25923 and *Listeria monocytogenes* ATCC19112) and two Gram-negative bacteria (*Salmonella typhimurium* ATCC23564 and *Escherichia coli* NCIM2931) by agar disk diffusion method[21]. The microorganisms were obtained from NCL, Pune,

India. The microorganisms were maintained at 4°C. Molten Mueller Hinton agar (40-42°C) was seeded with 200 µL of inoculums (1×10^8 CFU mL^{-1}) and poured into Petri dishes. The 20 µL of 10 mg mL^{-1} CuO NPs in 100% DMSO was loaded on a sterile disk. The plates were incubated at 37°C for 24 hrs. DMSO was used as a negative control. Antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the disc and the diameter was measured in millimeters. The experiment was done in triplicate and the average values were calculated for antibacterial activity.

Statistical analysis: All analyses were carried out in triplicates. Data were presented as mean ± standard error of mean using MS Excel 2016.

RESULTS AND DISCUSSION

Synthesis of CuO Nps: During the synthesis of CuO NPs, a color change was observed to the brownish color of the reaction mixture after the addition of leaf extract, indicating the presence of the nanoparticles (Fig. 1). Similar color change was observed in *Cissus vitiginea*[22] and *Cardiospermum halicacabum*[23].

Characterization of CuO NPs

UV-Vis spectroscopy: The UV-Vis spectroscopy is a fundamental technique to ascertain the formation of metal nanoparticles in aqueous solution. The UV-Visible absorption spectrum was used for the analysis of the optical properties of green synthesized CuO NPs. Initially synthesized CuO NPs were analysed by UV-Vis absorption spectroscopy to study their surface plasmon resonance (SPR). The UV-Vis absorption spectrum sample was done in a Shimadzu UV-1800. The UV-Vis spectrum of the reaction medium in leaf extract with 1 mM $CuSO_4 \times 5H_2O$ was recorded at 200-400 nm (Fig. 2). The reduction of the extract was confirmed by absorptivity peak at 339 nm observed in the UV Vis spectroscopy record as indicated by the red line and sign in the Fig. 2. This peak can be assigned to synthesized copper nanoparticles using plant extract. A broadened SPR peak was observed in this UV-Vis spectrum confirming that poly-dispersed nanosized particle is synthesized[22]. Thiruvengadam *et al.*[4] found a maximum absorption peak at 378 nm in *Millettia pinnata*-mediated CuNPs, however, Pérez-Alvarez *et al.*[24] observed a maximum peak at 340 nm in *Gossypium hirsutum* synthesized CuNPs.

Fourier-Transform Infrared Spectroscopy (FTIR): The FTIR analysis is used to identify and get approximate ideas of possible biomolecules that are responsible for the capping and stabilization of the CuO NPs with *P. pinnata* leaf extract. Investigation of the interaction between different species and the chemical composition of the mixtures was done using FTIR Spectroscopy. The spectra of CuO NPs revealed strong bonds at 3529.80, 3399.3, 3377.0, 3296.8, 2918.5, 2851.4, 2208.4, 2104.1, 2051.9, 2018.4, 1640.0, 1545.0, 1461.1, 1407.1, 1265.4,

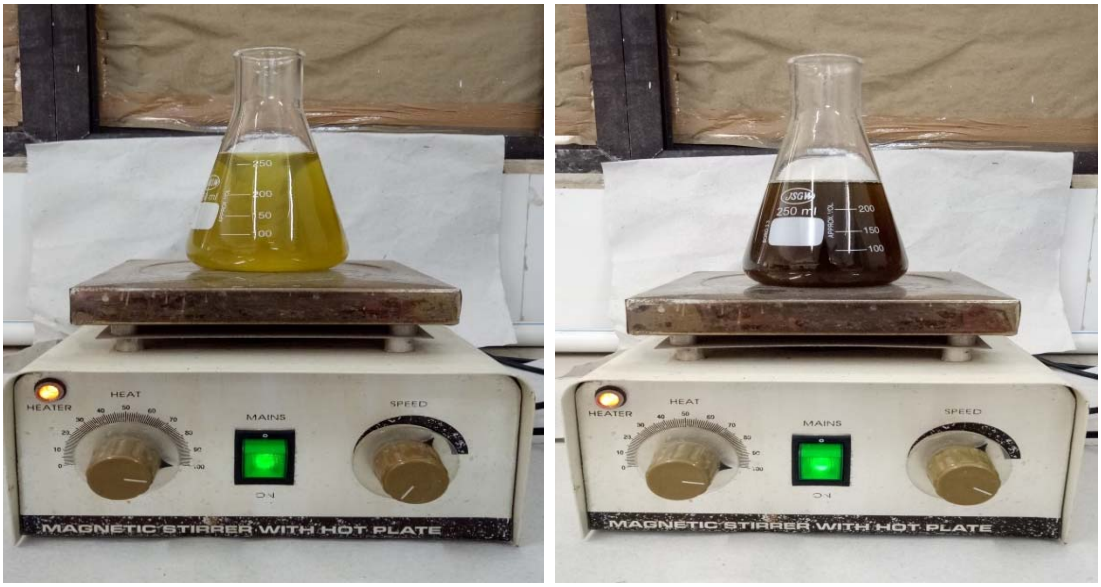

Fig. 1: Color change in the reaction mixture indicates formation of CuO Nps

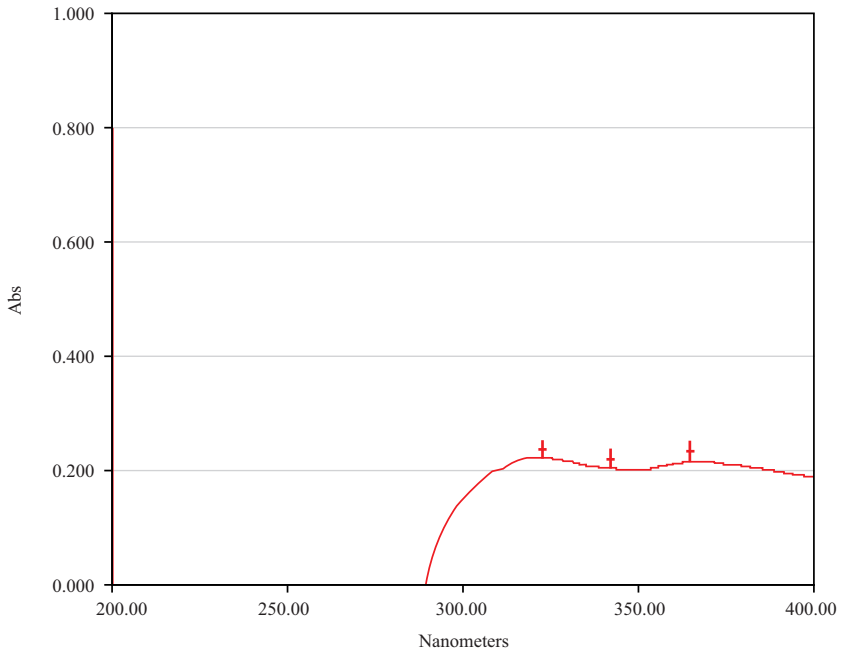

Fig. 2: UV-Vis spectrum of green synthesized CuO NPs

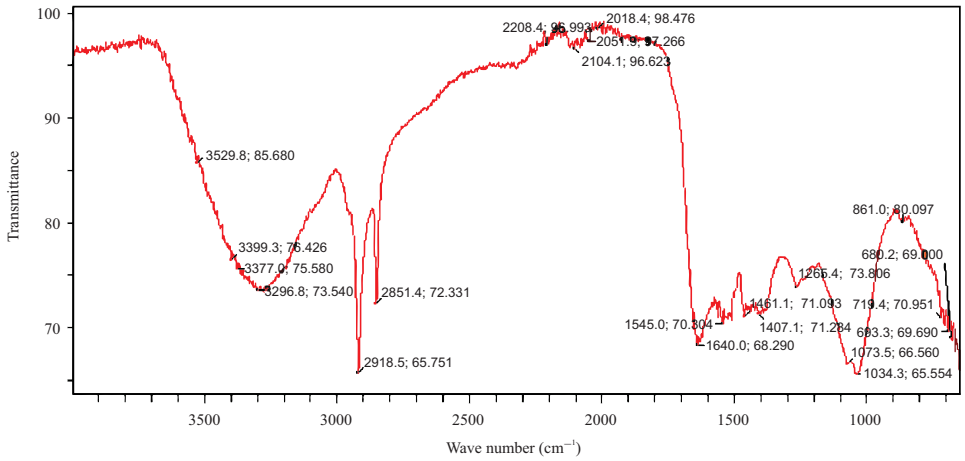

Fig. 3: FTIR spectrum of green synthesized CuO NPs

1073.5, 1034.3, 861.0, 719.4, 693.3 and 680.2 cm^{-1} (Fig. 3). Different frequency, bond and functional groups of CuO NPs was given in Table 1. Based on this observation it is clear that plant phytochemical and secondary metabolites are playing a key in the reduction of CuO NPs. Several authors[23,25,26] reported similar function groups like amine, alcohol, nitro compound, alkene and alkyne compound, which responded and acted as reducing and stabilizing agents for the synthesis of CuO Nps.

Scanning electron microscopy (SEM): The SEM analysis was employed to determine the particle size and morphology of the synthesized CuO NPs using *P. pinnata* extract at different magnifications were shown in Fig. 4(a-b). The CuO NPs were found to be irregular in shape. The particle size ranged between 40.3-101.2 nm and the average size of nanoparticles was 58.1 nm. Rajeshkumar *et al.*[12] found an average size range from 60-90 nm in *Cissus arnottiana* leaf-synthesized CuNPs. While Nagar and Devra[27] observed particle size 35-102 nm in *Azadirachta indica* synthesized CuNPs.

Zeta potential analysis: Zeta potential gives critical information about colloid stability. The particle size and zeta potential were found in the 40.3-101.2 nm range and

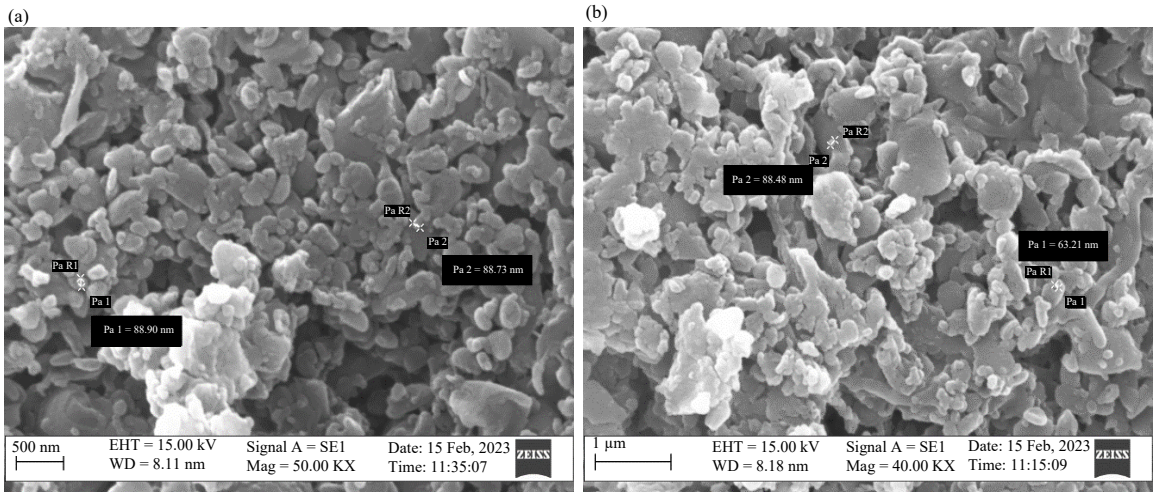

Fig. 4(a-b): SEM images of green synthesized CuO NPs at different magnifications (a) Mag: 50.00 KX and (b) 40.00 KX

Table 1: Frequency, bond and functional group of FTIR spectrum of green synthesized CuO NPs

Frequency	Bond	Functional group
3529.8	O-H stretching (M)	Alcohol
3399.3	N-H stretching (M)	Aliphatic primary amine
3377.0	N-H stretching (M)	Aliphatic primary amine
3296.8	C-H stretching (S)	Alkyne
2918.5	C-H stretching (M)	Alkane
2851.4	C-H stretching (M)	Alkane
2208.4	C≡N stretching (W)	Nitrile
2104.1	C≡C stretching (W)	Alkyne
2051.9	N = C = S stretching (S)	Isothiocyanate
2018.4	N = C = S stretching (S)	Isothiocyanate
1640.0	C = N stretching (M)	Imine/oxime
1545.0	N-O stretching (S)	Nitro compound
1461.1	C-H bending (M)	Alkane
1407.1	S = O stretching (S)	Sulphate
1265.4	C-O stretching (S)	Alkyl aryl ether
1073.5	C-O stretching (S)	Primary alcohol
1034.3	C-O stretching (S)	Primary alcohol
861.0	C-Cl	Halogen
719.4	C-Cl	Halogen
693.3	C-Cl	Halogen
680.2	C-Cl	Halogen

Table 2: Antibacterial activity of CuO NPs against Gram-positive and Gram-negative bacteria

Bacteria	CuO NPs
Staphylococcus aureus ATCC25923	13.5±0.0
Listeria monocytogenes ATCC19112	11.5±0.29
Salmonella typhimurium ATCC23564	10.0±0.62
Escherichia coli NCIM2931	12.5±0.0

Values are presented as Mean±SEM (n = 3)

+0.14 mV, respectively. Optimized formulation was found to be CuO NPs with particle size 58.1 nm and zeta potential of +0.14 mV (Fig. 5).

Antibacterial activity: Antibacterial activity of green synthesized CuO NPs was evaluated with concentrations of 20 mg mL^{-1} against two gram positive bacteria and two gram negative bacteria using the Agar disc diffusion method as shown in Table 2. All four bacteria were inhibited by CuO NPs. However, the antibacterial activity was more against *S. aureus* followed by *E. coli*. While the lowest activity was against *S. typhimurium*. Daniel *et al*.[28] observed

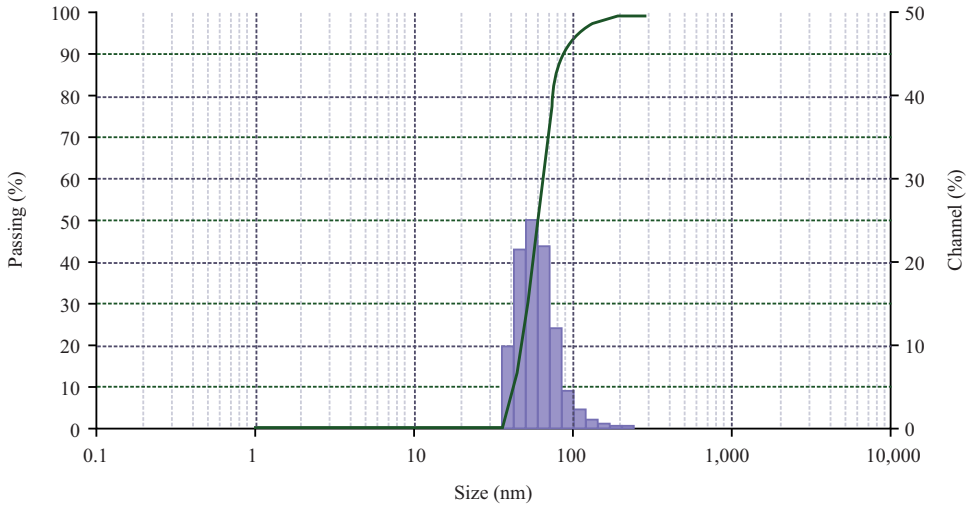

Fig. 5: Zeta potential graph of green synthesized CuO Nps
Mobility: 0.54 µ/s/V/cm, Zeta potential: 0.14 mV, Charge: 0.159 fC, Polarity: Positive and Conductivity: 222 µS cm^{-1}

Dodonaea viscosa extract synthesized CuNPs had remarkable antimicrobial activity. Usman *et al.*[29] observed copper nanoparticles plus chitosan synergistic antimicrobial activity against Gram-positive and Gram-negative bacteria.

The biosynthesized CuO NPs recommended using *P. pinnata* for further study related to antioxidant and anticancer activity as well as safety assessment.

CONCLUSION

The biosynthesis and characterization of CuO NPs using leaf extract of *P. pinnata* was performed and confirmed by spectroscopic and microscopic techniques. The average size of biosynthesized CuO NPs was 58.1 nm. The synthesized CuO NPs exhibited remarkable antibacterial activity. The output of this study suggested that synthesized CuO NPs have remarkable potential in the preparation of drugs used in therapeutic applications, especially against infectious diseases.

SIGNIFICANCE STATEMENT

Medicinal plants have been used for the synthesis of metal nanoparticles, due to easier availability, affordability and notable presence of bioactive secondary metabolites promotes rapid synthesis of the nanoparticles. Nowadays, a big challenge is bacterial resistance due to the overuse of the antibiotics. From this point of view, present study revealed the usage of the plant extract as natural source for the biosynthesis of the nanomaterial as metal oxide nanoparticles for the therapeutic application against the drug resistant problem caused by pathogenic microorganisms. Antibacterial efficacy of the copper oxide nanoparticles using *Pongamia pinnata* evaluated. In addition, characterisation of the nanoparticles employed using various analytical techniques. This study revealed the potent antibacterial efficacy of the copper oxide nanoparticles recommended for the further pharmacological activity and therapeutic applications.

REFERENCES

1. Khan, P.P., G. Talaviya, S. Vadavariya, H. Desai and V. Togadiya *et al.*, 2024. Evaluation of Antimicrobial, Antioxidant and Photocatalytic Activity of Zinc Oxide Nanoparticles Synthesized from *Parmotrema perlatum*. In: Nanotechnology and *in silico* Tools, Kaneria, M., K. Rakholiya and C. Egbuna (Eds.), Elsevier, Amsterdam, Netherlands, ISBN: 978-0-443-15457-7, pp: 141-154.
2. Mohanpuria, P., N.K. Rana and S.K. Yadav, 2008. Biosynthesis of nanoparticles: Technological concepts and future applications. J. Nanopart. Res., 10: 507-517.
3. Monica, R.C. and R. Cremonini, 2009. Nanoparticles and higher plants. Caryologia, 62: 161-165.
4. Thiruvengadam, M., I.M. Chung, T. Gomathi, M.A. Ansari, V.G. Khanna, V. Babu and G. Rajakumar, 2019. Synthesis, characterization and pharmacological potential of green synthesized copper nanoparticles. Bioprocess Biosyst. Eng., 42: 1769-1777.
5. Valan, S.L., A.E. de Cruz, P.J. Jacob and S. Djearamane, 2022. Sustainable synthesis of copper oxide nanoparticles using *Aquilaria malaccensis* (Agarwood) leaf extract as reducing agent. Int. J. Technol., 13: 1115-1125.

6. Hamad, A.M., Q.M. Atiyea, D.N. Abdul Hameed and A.H. Dalaf, 2023. Green synthesis of copper nanoparticles using strawberry leaves and study of properties, anti-cancer action, and activity against bacteria isolated from COVID-19 patients. Karbala Int. J. Mod. Sci., Vol. 9. 10.33640/2405-609X.3275.
7. Hassanien, R., D.Z. Husein and M.F. Al-Hakkani, 2018. Biosynthesis of copper nanoparticles using aqueous *Tilia* extract: Antimicrobial and anticancer activities. Heliyon, Vol. 4. 10.1016/j.heliyon.2018.e01077.
8. Dashtizadeh, Z., F.J. Kashi and M. Ashrafi, 2021. Phytosynthesis of copper nanoparticles using *Prunus mahaleb* L. and its biological activity. Mater. Today Commun., Vol. 27. 10.1016/j.mtcomm.2021.102456.
9. Tahvilian, R., M.M. Zangeneh, H. Falahi, K. Sadrjavadi and A.R. Jalalvand *et al*., 2019. Green synthesis and chemical characterization of copper nanoparticles using *Allium saralicum* leaves and assessment of their cytotoxicity, antioxidant, antimicrobial, and cutaneous wound healing properties. Appl. Orga. Chem., Vol. 33. 10.1002/aoc.5234.
10. Das, P.E., I.A. Abu-Yousef, A.F. Majdalawieh, S. Narasimhan and P. Poltronieri, 2020. Green synthesis of encapsulated copper nanoparticles using a hydroalcoholic extract of *Moringa oleifera* leaves and assessment of their antioxidant and antimicrobial activities. Molecules, Vol. 25. 10.3390/molecules25030555.
11. Rajagopal, G., A. Nivetha, M. Sundar, T. Panneerselvam and S. Murugesan *et al*., 2021. Mixed phytochemicals mediated synthesis of copper nanoparticles for anticancer and larvicidal applications. Heliyon, Vol. 7. 10.1016/j.heliyon.2021.e07360.
12. Rajeshkumar, S., S. Menon, S.V. Kumar, M.M. Tambuwala and H.A. Bakshi *et al*., 2019. Antibacterial and antioxidant potential of biosynthesized copper nanoparticles mediated through *Cissus arnotiana* plant extract. J. Photochem. Photobiol. B: Biol., Vol. 197. 10.1016/j.jphotobiol.2019.111531.
13. Thakur, S., H. Kaurav and G. Chaudhary, 2021. Karanj (*Pongamia pinnata*)-An ayurvedic and modern overview. Asian J. Pharm. Clin. Res., 6: 14-21.
14. Wen, R., H.N. Lv, Y. Jiang and P.F. Tu, 2019. Anti-inflammatory pterocarpanoids from the roots of *Pongamia pinnata*. J. Asian Nat. Prod. Res., 21: 859-866.
15. Tamrakar, A.K., P.P. Yadav, P. Tiwari, R. Maurya and A.K. Srivastava, 2008. Identification of pongamol and karanjin as lead compounds with antihyperglycemic activity from *Pongamia pinnata* fruits. J. Ethnopharmacol., 118: 435-439.
16. Badole, S.L., A.A. Zanwar, A.E. Ghule, P. Ghosh and S.L. Bodhankar, 2012. Analgesic and anti-inflammatory activity of alcoholic extract of stem bark of *Pongamia pinnata* (L.) Pierre. Biomed. Aging Pathol., 2: 19-23.
17. Ramdhave, A.S., S.L. Badole and S.L. Bodhankar, 2011. Anticonvulsant activity of stem bark of *Pongamia pinnata*. Biomed. Aging Pathol., 1: 147-157.
18. Badole, S.L. and S.L. Bodhankar, 2010. Antidiabetic activity of cycloart-23-ene-3β, 25-diol (B2) isolated from *Pongamia pinnata* (L. Pierre) in streptozotocin-nicotinamide induced diabetic mice. Eur. J. Pharmacol., 632: 103-109.
19. Dwivedi, D., M. Dwivedi, S. Malviya and V. Singh, 2017. Evaluation of wound healing, anti-microbial and antioxidant potential of *Pongamia pinnata* in wistar rats. J. Tradit. Complementary Med., 7: 79-85.
20. Kaneria, M.J., K.D. Rakholiya, L. Marsonia, R.A. Dave and B.A. Golakiya, 2018. Nontargeted metabolomics approach to determine metabolites profile and antioxidant study of tropical almond (*Terminalia catappa* L.) fruit peels using GC-QTOF-MS and LC-QTOF-MS. J. Pharm. Biomed. Anal., 160: 415-427.
21. Rakholiya, K. and S. Chanda, 2012. *In vitro* interaction of certain antimicrobial agents in combination with plant extracts against some pathogenic bacterial strains. Asian Pac. J. Trop. Biomed., 2: S876-S880.
22. Wu, S., S. Rajeshkumar, M. Madasamy and V. Mahendran, 2020. Green synthesis of copper nanoparticles using *Cissus vitiginea* and its antioxidant and antibacterial activity against urinary tract infection pathogens. Artif. Cells, Nanomed. Biotechnol., 48: 1153-1158.
23. Punniyakotti, P., P. Panneerselvam, D. Perumal, R. Aruliah and S. Angaiah, 2020. Anti-bacterial and anti-biofilm properties of green synthesized copper nanoparticles from *Cardiospermum halicacabum* leaf extract. Bioprocess Biosyst. Eng., 43: 1649-1657.
24. Pérez-Alvarez, M., G. Cadenas-Pliego, O. Pérez-Camacho, V.E. Comparán-Padilla, C.J. Cabello-Alvarado and E. Saucedo-Salazar, 2021. Green synthesis of copper nanoparticles using cotton. Polymers, Vol. 13. 10.3390/polym13121906.
25. Jahan, I., F. Erci and I. Isildak, 2021. Facile microwave-mediated green synthesis of non-toxic copper nanoparticles using *Citrus sinensis* aqueous fruit extract and their antibacterial potentials. J. Drug Delivery Sci. Technol., Vol. 61. 10.1016/j.jddst.2020.102172.
26. Zebari, O.I.H., S.Y.S. Zeebaree, A.Y.S. Zeebaree, H.I.H. Zebari and H.R. Abbas, 2019. Antibacterial activity of copper nanoparticles fabricate via *Malva sylvesteris* leaf extract. Kurdistan J. Appl. Res., 4: 146-156.
27. Nagar, N. and V. Devra, 2018. Green synthesis and characterization of copper nanoparticles using *Azadirachta indica* leaves. Mater. Chem. Phys., 213: 44-51.
28. Daniel, S.C.G.K., G. Vinothini, N. Subramanian, K. Nehru and M. Sivakumar, 2013. Biosynthesis of Cu, ZVI, and Ag nanoparticles using *Dodonaea viscosa* extract for antibacterial activity against human pathogens. J. Nanopart. Res., Vol 15. 10.1007/s11051-012-1319-1.
29. Usman, M.S., M.E. El Zowalaty, K. Shameli, N. Zainuddin, M. Salama and N.A. Ibrahim, 2013. Synthesis, characterization and antimicrobial properties of copper nanoparticles. Int. J. Nanomed., 8: 4467-4479.