Journal of Advances in Biology & Biotechnology

Volume 27, Issue 11, Page 493-504, 2024; Article no.JABB.126395
ISSN: 2394-1081

Characterization of Copper Nanoparticles Synthesized Using the Leaf Extracts of *Lawsonia inermis*, *Eucalyptus globulus*, *Psidium guajava*, and *Azadirachta indica*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SS, HH, and AP conducted the research, author SJM supervised the work. Author MKJ wrote, reviewed, and edited the manuscript. All authors have read and approved the manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i111634

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/126395

Original Research Article

Received: 03/09/2024
Accepted: 05/11/2024
Published: 09/11/2024

ABSTRACT

Green synthesis of nanoparticles using plant extracts is a sustainable, eco-friendly, non-toxic, and cost-efficient approach. In this study, copper nanoparticles (CuNPs) were synthesized using the mehandi *Lawsonia inermis*, nilgiri *Eucalyptus globulus*, guava *Psidium guajava*, or neem

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Cite as: *Sahu, S., S.J. Magar, M. K. Jena, H. Hakeem, and A. Padhi. 2024. "Characterization of Copper Nanoparticles Synthesized Using the Leaf Extracts of Lawsonia Inermis, Eucalyptus Globulus, Psidium Guajava, and Azadirachta Indica". Journal of Advances in Biology & Biotechnology 27 (11):493-504. https://doi.org/10.9734/jabb/2024/v27i111634.*

Azadirachta indica leaf extracts and 1mM $CuSO_4.5H_2O$ salt solution in a ratio of 1:3 at 80^0C temperature and characterized using UV-Visible spectroscopy, Fourier Transform Infrared spectroscopy, and Field Emission Scanning Electron Microscopy analysis. All the CuNPs showed colour change with characteristic surface plasmon resonance bands. The plasmon resonance bands of *L. inermis*, *P. guajava,* and *A. indica* CuNPs peaked at 300nm while those of *E. globulus* CuNPs peaked at 400nm. The CuNPs synthesized from *L. inermis*, *E. globulus*, and *P. guajava* leaf extracts exhibited brown colour while those synthesized from *A. indica* leaf extracts showed green colour. Eleven bands were present in *L. inermis* and *A. indica* CuNPs in the range of 538.61-3649.06cm^{-1} and 3867.26-519.02cm^{-1}, respectively, while ten were in the *E. globulus* and *P. guajava* CuNPs in the range of 407.25-3326.78cm^{-1} and 3325.93-407.07cm^{-1}, respectively. The *L. inermis*, *E. globulus*, and *A. indica* CuNPs were spherical, except *P. guajava* CuNPs which were flaky in shape. The particle size of *L. inermis* CuNPs was found in the range of 8.20 to 15.94nm; *E. globulus* CuNPs in the range of 7.52 to 67.47nm; *P. guajava* CuNPs in the range of 18.90 to 73.80nm and *A. indica* CuNPs in the range of 9.86 to 22.05nm.

Keywords: Field emission scanning electron microscopy; fourier transform infrared spectroscopy; guava; mehandi; neem; nilgiri; UV-Visible spectral analysis.

1. INTRODUCTION

Nanotechnology involves manipulating matter at the atomic and molecular scale to create new materials and devices, with applications in fields like agriculture, medicine, cosmetics, food science, and energy (Kingsley et al. 2013). The metal nanoparticles' small size and unique physical and chemical properties make them useful in various agricultural sectors (Kingsley et al. 2013). Traditional physical and chemical methods for synthesizing metal nanoparticles have several limitations, such as labour-intensive, environmentally challenging, high production cost, and long synthesis and purification times (Nagajyothi & Lee 2011). However, green synthesis of metal nanoparticles using plant extracts, algae, fungi, and microbial enzymes offers a sustainable, non-toxic, and efficient approach to nanotechnology (Castro et al. 2013, Singh et al. 2016, Bartolucci et al. 2020, Nirmala et al. 2020, Bahrulolum et al. 2021, Padhi et al. 2024). This eco-friendly method leverages biomolecules found in plants to aid in the creation of stable metal nanoparticles (Vaghari et al. 2026). The metal nanoparticles help to increase the communication between plant roots and the surrounding soil structure (Dhillon & Mukhopadhyay 2015). Copper nanoparticles (CuNPs) are gaining attention due to their cost-effectiveness, high electrical conductivity, and antifungal properties, making them useful in industries such as medicine, electronics, and agriculture (Johnson 1935 and Saran et al. 2018). This study aimed to characterize the CuNPs synthesized using the leaf extracts of mehandi *Lawsonia inermis* L., nilgiri *Eucalyptus globulus* Labill, guava *Psidium guajava* L., or neem *Azadirachta indica* A. Juss, and 1mM $CuSO_4.5H_2O$ salt solution in a ratio of 1:3 at 80^0C temperature.

2. MATERIALS AND METHODS

The experiment was conducted from November 2023 to June 2024 in the Department of Plant Pathology, College of Agriculture, Latur, Maharashtra, India.

2.1 Green Synthesis of Copper Nano-particles

Copper nanoparticles were synthesized following the protocol given by Padhi et al. (2024) with slight modifications. To synthesize CuNPs, copper sulphate ($CuSO_4 \cdot 5H_2O$) salt and leaf extracts of *L. inermis*, *E. globulus*, *P. guajava,* or *A. indica* plants were used as precursors. The fresh leaves of *L. inermis*, *E. globulus*, *P. guajava,* and *A. indica* plants were collected from the premises of the College of Agriculture, Latur, Maharashtra, India, and used for preparing leaf extracts. One hundred grams of roughly fresh leaves of each plant were taken, cut, and boiled separately with 100ml of distilled water for 15min using a water bath at 80°C. The leaf extracts were filtered and stored in a refrigerator. The CuNPs were synthesized using the leaf extracts of each plant separately. Leaf extracts were added to an aqueous 1mM concentration of $CuSO_4 \cdot 5H_2O$ solution in a 1:3 ratio at 80^oC temperature and kept for 60min of incubation period in the magnetic stirrer. The solutions, thus obtained were purified by repeated centrifugation at 5,000 rotations per minute (rpm) for 15min. The solution formed showed the change in colour

from light brown to dark brown in the case of *L. inermis*, *E. globulus*, and *P. guajava* extracts as reducing agents and the change from light green to dark green in the case of *A. indica* extracts (Fig. 1), confirming the formation of CuNPs. The obtained CuNPs were dried using a hot air oven at 80°C for 3h. The dried particles were then ground into fine powders and stored at room temperature for further analysis.

2.2 Characterization of Copper Nano-particles

UV-Visible (UV-Vis) spectral analysis, Field Emission Scanning Electron Microscopy (FE-SEM), and Fourier Transform Infrared (FTIR) spectroscopy of CuNPs were performed following the protocol given by Padhi et al. (2024). UV-Vis spectral analysis was performed using a UV-Vis spectrophotometer at the Vilasrao Deshmukh College of Agricultural Biotechnology, Latur, Maharashtra, India. The reduction of pure Cu+ ions was monitored by recording the UV-Vis spectrum of the reaction medium after diluting a small aliquot of the sample into deionized water and subsequently analyzed at room temperature between 200-800nm.

The CuNPs were characterized by performing FE-SEM to determine their size and shape at the Connecting Research Centre, Bhubaneswar, Odisha, India. To characterize the specimens in this study, Zeiss Crossbeam 340 was used to capture the microstructure image. In FE-SEM analysis, electrons were released from a field emission source and the generated primary electrons were accelerated in a high electrical field gradient. The primary electrons were focused and deflected by electronic lenses and produced a narrow scan beam that bombarded the object (CuNPs) within the high vacuum column. The secondary electrons containing the sample information were caught by the detector and produced an electronic signal. Then, the signal was amplified and transformed into a digital image.

Fig. 1. Copper nanoparticle solutions (A) *Psidium guajava* CuNPs, (B) *Eucalyptus globulus* CuNPs, (C) *Azadirachta indica* CuNPs, (D) *Lawsonia inermis* CuNPs

The FTIR- spectroscopy was conducted at the Central Instrumentation Facility, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India. For FTIR Spectroscopy measurements, the bio-transformed products present in cell-free filtrate after 72h of incubation were freeze-dried and diluted with potassium bromide in the ratio of 1:100. FTIR spectrum of CuNPs was recorded on FTIR instrument mode using Nicolet 6700 spectrometer of resolution of 4cm^{-1} attachment. All measurements were carried out in the range of 500cm^{-1} to 4000cm^{-1} at a resolution of 4cm^{-1}.

3. RESULTS AND DISCUSSION

The results of UV-Vis spectroscopy presented in Fig. 2 revealed characteristic surface plasmon resonance bands as indicative of CuNPs formation. All the synthesized CuNPs showed colour change with characteristic surface plasmon resonance bands. The plasmon resonance bands of CuNPs synthesized using the leaf extracts of *L. inermis*, *E. globulus*, *P. guajava,* and *A. indica* peaked at 300nm, 400nm, 300nm, and 300nm, respectively. The UV-Vis spectral analysis confirmed the formation and stability of CuNPs in aqueous solution. CuNPs synthesized from *L. inermis*, *E. globulus*, and *P. guajava* exhibited brown colour while those synthesized from *A. indica* leaf extracts showed green colour due to excitation of surface plasmon vibrations, confirming the reduction of copper ions extracellularly. These findings are similar to those of Thube et al. (2023) who observed the UV absorption peaks of *L. inermis* CuNPs at 296nm and *A. indica* CuNPs at 300nm. However, the present findings are deviated from those of Upadhyaya et al. (2018) who observed that *L. inermis* ZnNPs showed an absorption peak at 385nm which might be due to different metal salts or their concentrations used for synthesis. The *E. globulus* CuNPs showed an absorption peak at 345nm (Dhole et al. 2024)*; P. guajava* CuNPs at 294nm (Caroling et al. 2015) and *A. indica* CuNPs at 350nm (Gurudevi et al. 2023). which deviated from the present findings. The deviation might be associated with the differences in the methods of synthesis, chemical agents, and their concentration used for the synthesis of CuNPs.

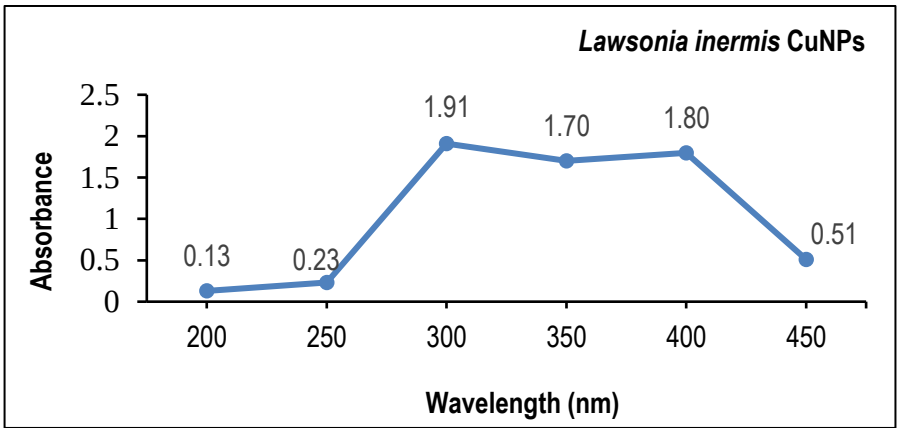
The FTIR spectrum of *L. inermis* CuNPs in Table 1 and depicted in Fig. 3 showed the presence of eleven bands, such as O-H stretching alcohol (medium and sharp bond, 3649.06cm^{-1} wave number, and 99.25% transmittance), O-H stretching alcohol (medium and sharp bond, 3611.07cm^{-1} wave number, and 99.09% transmittance), O-H stretching alcohol (strong and broad bond, 3245.65cm^{-1} wave number, and 97.42% transmittance), C≡C stretching alkyne (weak and disubstituted bond, 2186.13cm^{-1} wave number, and 99.19% transmittance), N=C=S stretching isothiocyanate (strong bond, 2054.13cm^{-1} wave number, and 99.15% transmittance), N=C=S stretching isothiocyanate (strong bond, 2038.69cm^{-1} wave number, and 99.15% transmittance), C-H bending aromatic compound (1843.15cm^{-1} wave number and 99.07% transmittance), COO-H bending carboxylic acid (medium bond, 1396.43cm^{-1} wave number, and 96.80% transmittance), S=O stretching sulfoxide (strong bond, 1033.53cm^{-1} wave number, and 92.99% transmittance), C=C bending alkene (medium and trisubstituted bond, 816.35cm^{-1} wave number, and 96.11% transmittance), and C-I stretching halo compound (strong bond, 538.61cm^{-1} wave number, and 93.39% transmittance).

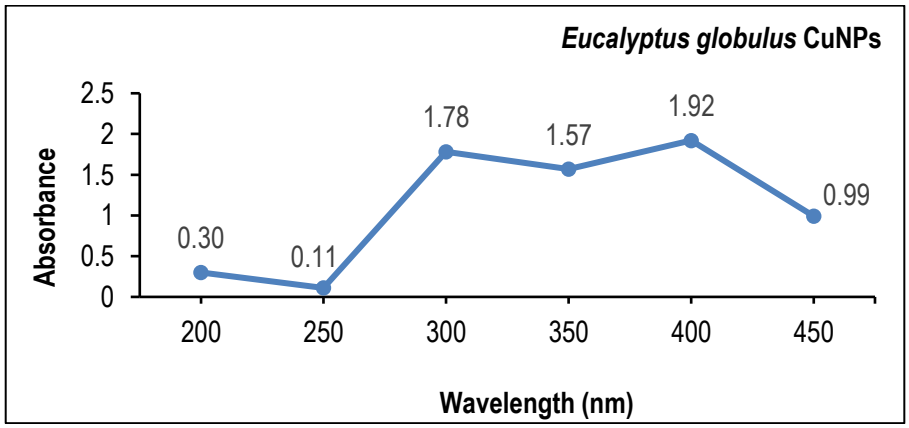
The FTIR spectrum of *E. globulus* CuNPs presented in Table 1 and depicted in Fig. 3 showed the presence of ten bands at wave numbers, 3326.78cm^{-1} (49.27% transmittance, O-H stretching intermolecular bonded alcohol, strong and broad bond), 2122.01cm^{-1} (95.72% transmittance, N=N=N stretching azide, strong bond), 1635.03cm^{-1} (70.71% transmittance, C=C stretching conjugated alkene, medium bond), 1053.09cm^{-1} (89.46% transmittance, C=O stretching ketone, strong bond), and 1033.17 cm^{-1} (88.61% transmittance, S=O stretching sulfoxide, strong bond). The presence of a C-I stretching halo compound strong bond was confirmed by bands at 486.72cm^{-1} (32.88% transmittance), 446.89cm^{-1} (31.73% transmittance), 426.53cm^{-1} (31.05% transmittance), 417.95cm^{-1} (31.15% transmittance), and 407.25cm^{-1} (31.29% transmittance), respectively.

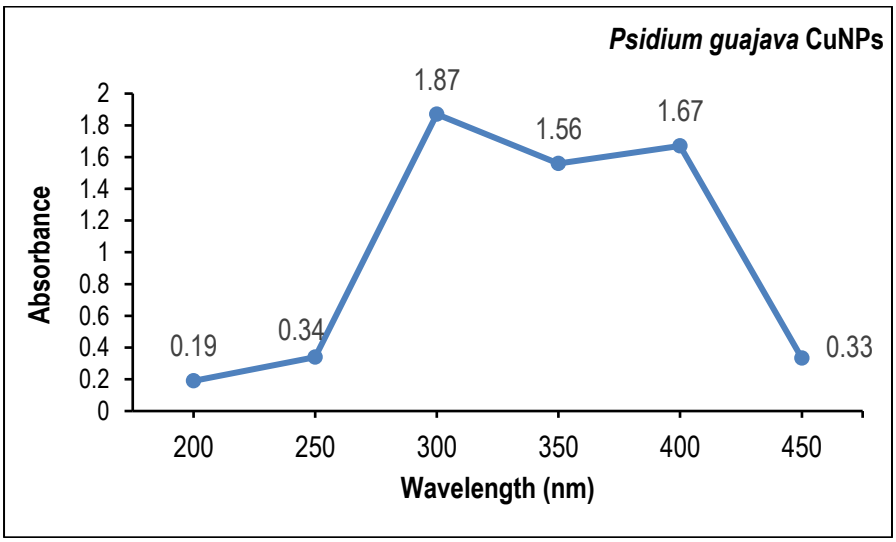
The FTIR spectrum of *P. guajava* CuNPs presented in Table 1 and depicted in Fig. 3 showed the presence of ten bands at wave numbers 3325.93cm^{-1} (49.35% transmittance, N-H stretching secondary amine, medium bond), 2160.38cm^{-1} (95.81% transmittance, S-C≡N stretching thiocyanate, strong bond), 2106.30cm^{-1} (95.62% transmittance, N=C=S stretching isothiocyanate, strong bond), 1634.85cm^{-1} (70.76% transmittance, C=C stretching conjugated alkene, medium bond), 1054.20cm^{-1} (89.53% transmittance, C=O stretching ketone,

strong bond), and 1033.19cm^{-1} (88.78% transmittance, S=O stretching sulfoxide, strong bond). The presence of C-I stretching halo compound strong bonds was confirmed by bands at wave numbers 449.89cm^{-1} (31.89% transmittance), 435.08cm^{-1} (31.68% transmittance), 418.80cm^{-1} (31.20% transmittance), and 407.07cm^{-1} (31.65% transmittance), respectively.

The FTIR spectrum of *A. indica* CuNPs presented in Table 1 and depicted in Fig. 3







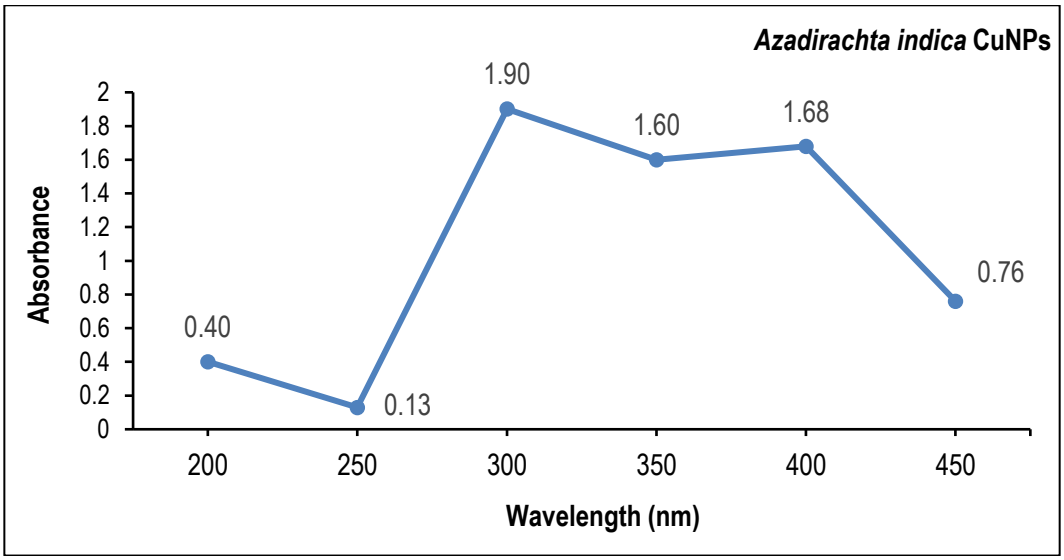


Fig. 2. UV-Visible spectra recorded after exposure of 1mM CuSO4.5H2O solution in the leaf extracts of *Lawsonia inermis, Eucalyptus globulus, Psidium guajava,* and *Azadirachta indica*

revealed the presence of eleven bands at wave numbers 3867.26cm^{-1} (98.93% transmittance, O-H stretching alcohol, medium and sharp bond), 3649.07cm^{-1} (98.79% transmittance, O-H stretching alcohol, medium and sharp bond), 3254.97cm^{-1} (94.99% transmittance, N-H stretching aliphatic primary amine, medium bond), 2917.44cm^{-1} (96.37% transmittance, CO-H stretching doublet aldehyde, medium bond), 2054.61cm^{-1} (98.43% transmittance, N=C=S stretching isothiocyanate, strong bond), 1956.34cm^{-1} (98.36% transmittance, C=C=C stretching allene, medium bond), 1597.68cm^{-1} (92.49% transmittance, N-H bending amine, medium bond), 1396.28 cm^{-1} (93.53% transmittance, COO-H bending carboxylic acid, medium bond), 1045.65cm^{-1} (85.80% transmittance, CO-O-CO stretching anhydride, strong and broad bond), 818.25cm^{-1} (91.89% transmittance, C=C bending alkene, medium and trisubstituted bond), and 519.02cm^{-1} (85.02% transmittance, C-I stretching halo compound, strong bond), respectively.

Similar findings were reported by Kolekar et al. (2015) in their FTIR analysis of CuNPs synthesized using *E. globulus* leaf extract, which showed bands within 500cm^{-1} to 3500cm^{-1} range. Caroling et al. (2015) reported that the FTIR spectrum of *P. guajava* CuNPs showed bands within the 1053cm^{-1} to 3419cm^{-1} range. Noorjahan (2020) reported that the FTIR spectrum of *L. inermis* FeNPs showed bands within the 1106cm^{-1} to 3414cm^{-1} range. Gopalakrishnan and Muniraj (2021) reported that the FTIR spectrum of *A. indica* CuNPs showed bands within the range of 622cm^{-1} to 3561cm^{-1}.

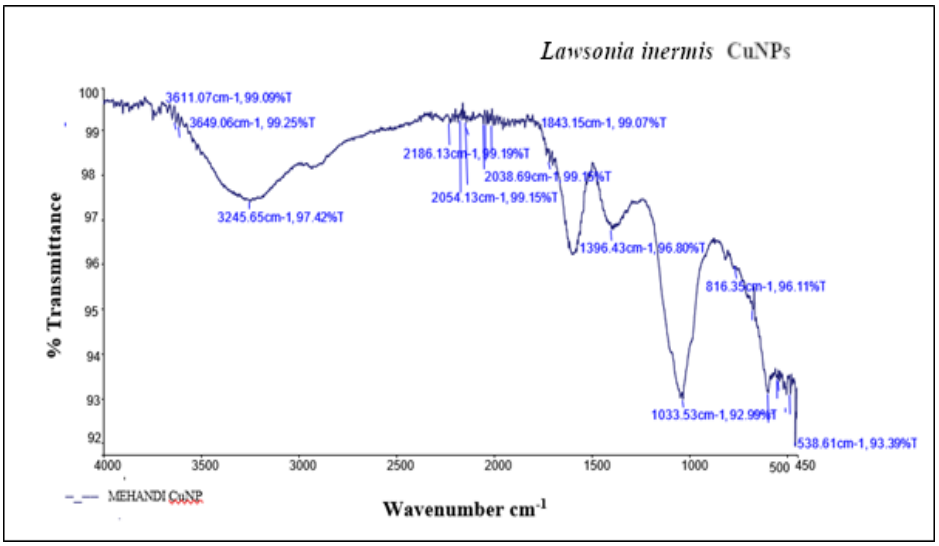
The image produced by FE-SEM in Fig. 4 showed individual CuNPs as well as several aggregates. The *L. inermis*, *E. globulus*, and *A. indica* CuNPs were spherical, except *P. guajava* CuNPs which were flaky in shape. Similarly, the spherical shape of *P. guajava* and *L. inermis* CuNPs was reported by Caroling *et al. (2015)* and Thube (2023) respectively while the quasi-spherical shape of *Eucalyptus* sp. CuNPs was observed by Alhalili (2022).

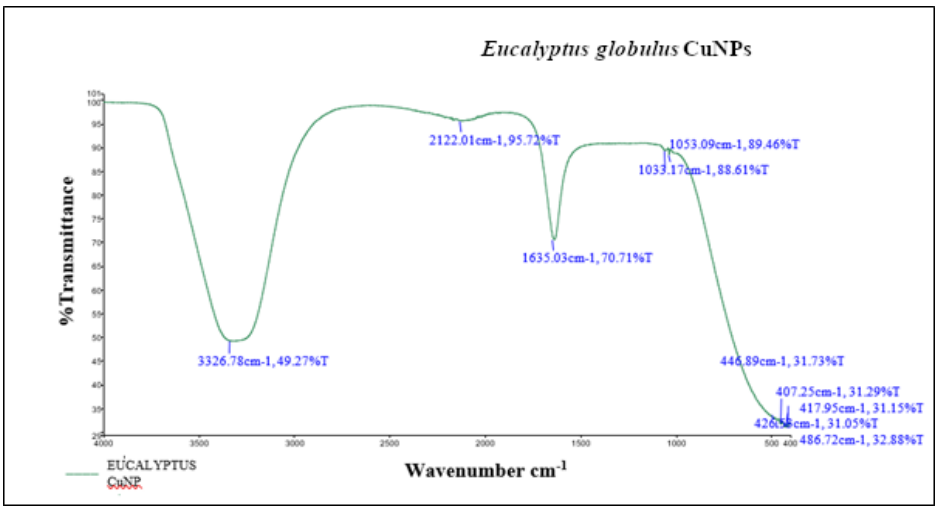
The FE-SEM revealed that the particle size of *L. inermis* CuNPs was found in the range of 8.20 to 15.94nm; *E. globulus* CuNPs in the range of 7.52 to 67.47nm; *P. guajava* CuNPs in the range of 18.90 to 73.80nm and *A. indica* CuNPs in the range of 9.86 to 22.05nm (Fig. 4). These findings are similar to those of Caroling et al. (2015) who reported that the particle size of *P. guajava* CuNPs was 15 to 30nm in diameter. However, the present findings are slightly deviated from those of Alhalili (2022) who found that the mean particle size of *Eucalyptus* sp. CuNPs were 88nm. The mean size of *A. indica* CuNPs was 48nm ((Nagar and Devra, 2018) and 5nm (Gopalakrishnan & Muniraj 2021). The size of *L. inermis* CuNPs was found in the range of 20.77 to 58.85nm (Thube 2023). The variation in the size of CuNPs might be due to the variation in the concentration of solutions used and the method of synthesis.

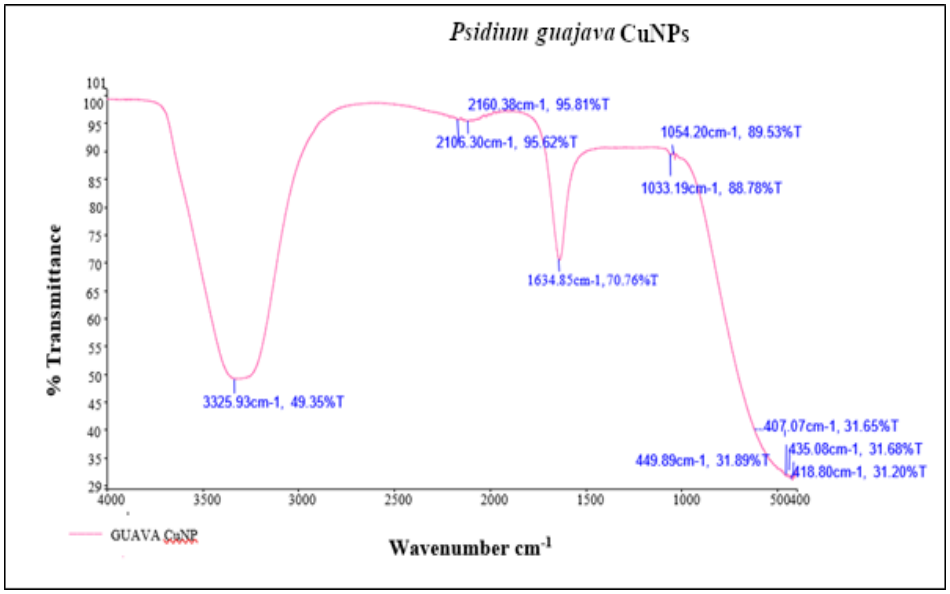
Table 1. The wave number (*v)* and transmittance (T) of functional groups of copper nanoparticles synthesized from the leaf extracts of *Lawsonia inermis*, *Eucalyptus globulus*, *Psidium guajava,* and *Azadirachta indica* resulted from the Fourier Transform Infrared spectroscopy analysis

***Lawsonia inermis* CuNps**			***Eucalyptus globulus* CuNps**			***Psidium guajava* CuNps**			***Azadirachta indica* CuNps**		
Functional group	***v* (cm^{-1})**	**T (%)**	**Functional group**	***v* (cm^{-1})**	**T (%)**	**Functional group**	***v* (cm^{-1})**	**T (%)**	**Functional group**	***v* (cm^{-1})**	**T (%)**
O-H	3649.06	99.25	O-H	3326.78	49.27	N-H	3325.93	49.35	O-H	3867.26	98.93
O-H	3611.07	99.09	N=N=N	2122.01	95.72	S-C≡N	2160.38	95.81	O-H	3649.07	98.79
O-H	3245.65	97.42	C=C	1635.03	70.71	N=C=S	2106.30	95.62	N-H*	3254.97	94.99
C≡C	2186.13	99.19	C=O	1053.09	89.46	C=C	1634.85	70.76	CO-H	2917.44	96.37
N=C=S	2054.13	99.15	S=O	1033.17	88.61	C=O	1054.20	89.53	N=C=S	2054.61	98.43
N=C=S	2038.69	99.15	C-I	486.72	32.88	S=O	1033.19	88.78	C=C=C	1956.34	98.36
C-H	1843.15	99.07	C-I	446.89	31.73	C-I s	449.89	31.89	N-H**	1597.68	92.49
COO-H	1396.43	96.80	C-I	426.53	31.05	C-I	435.08	31.68	COO-H*	1396.28	93.53
S=O	1033.53	92.99	C-I	417.95	31.15	C-I	418.80	31.20	CO-O-CO	1045.65	85.80
C=C*	816.35	96.11	C-I	407.25	31.29	C-I	407.07	31.65	C=C**	818.25	91.89
C-I	538.61	93.39	--	--	--	--	--	--	C-I	519.02	85.02

O-H: stretching alcohol, N-H: stretching secondary amine, N-H: stretching aliphatic primary amine, N-H**: bending amine, N=N=N: stretching azide, S-C≡N: stretching thiocyanate, N=C=S: stretching isothiocyanate, C-H: bending aromatic compound, C=C: stretching conjugated alkene, C=C*: bending alkene trisubstituted, C=C**: bending alkene, C=C=C: stretching allene, C≡C: stretching alkyne disubstituted, C-I: stretching halo compound, CO-H: stretching aldehyde, C=O stretching, ketone, COO-H: bending carboxylic acid, CO-O-CO: stretching anhydride, S=O: stretching sulfoxide*


Lawsonia inermis CuNPs
3611.07cm-1, 99.09%T
3649.06cm-1, 99.25%T
3245.65cm-1, 97.42%T
2186.13cm-1, 99.19%T
2038.69cm-1, 99.15%T
2054.13cm-1, 99.15%T
1843.15cm-1, 99.07%T
1396.43cm-1, 96.80%T
816.39cm-1, 96.11%T
1033.53cm-1, 92.99%T
538.61cm-1, 93.39%T
% Transmittance
Wavenumber cm-1
MEHANDI CuNP


Eucalyptus globulus CuNPs
2122.01cm-1, 95.72%T
1053.09cm-1, 89.46%T
1033.17cm-1, 88.61%T
1635.03cm-1, 70.71%T
3326.78cm-1, 49.27%T
446.89cm-1, 31.73%T
407.25cm-1, 31.29%T
417.95cm-1, 31.15%T
486.72cm-1, 32.88%T
%Transmittance
Wavenumber cm-1
EUCALYPTUS CuNP


Psidium guajava CuNPs
2160.38cm-1, 95.81%T
2106.30cm-1, 95.62%T
1054.20cm-1, 89.53%T
1033.19cm-1, 88.78%T
1634.85cm-1, 70.76%T
3325.93cm-1, 49.35%T
407.07cm-1, 31.65%T
435.08cm-1, 31.68%T
449.89cm-1, 31.89%T
418.80cm-1, 31.20%T
% Transmittance
Wavenumber cm-1
GUAVA CuNP

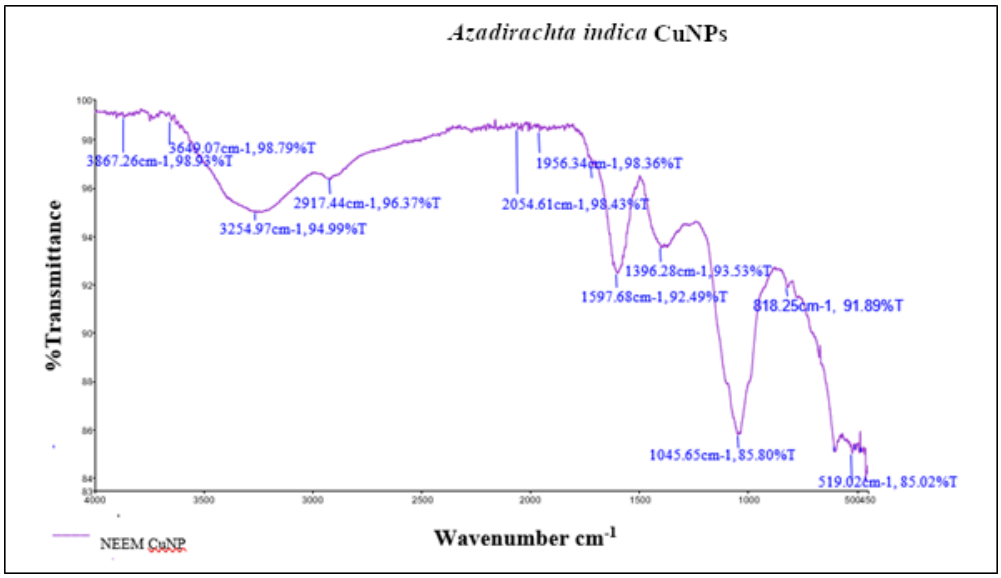
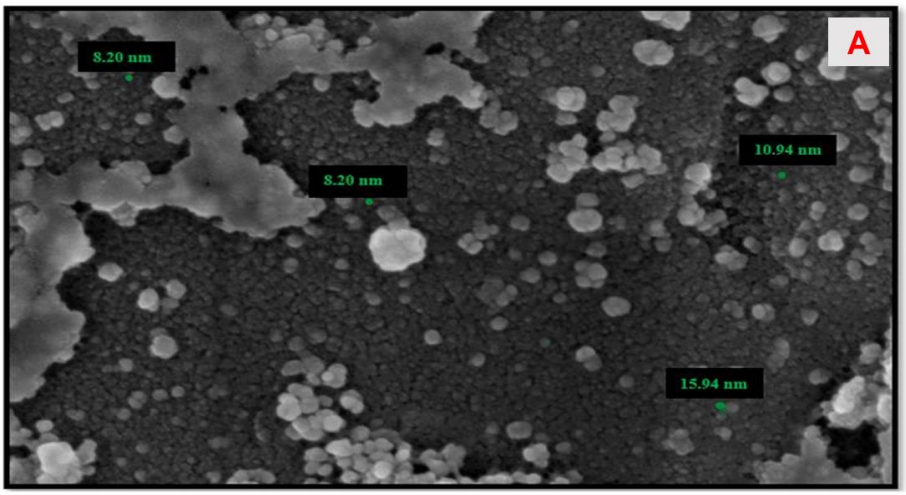
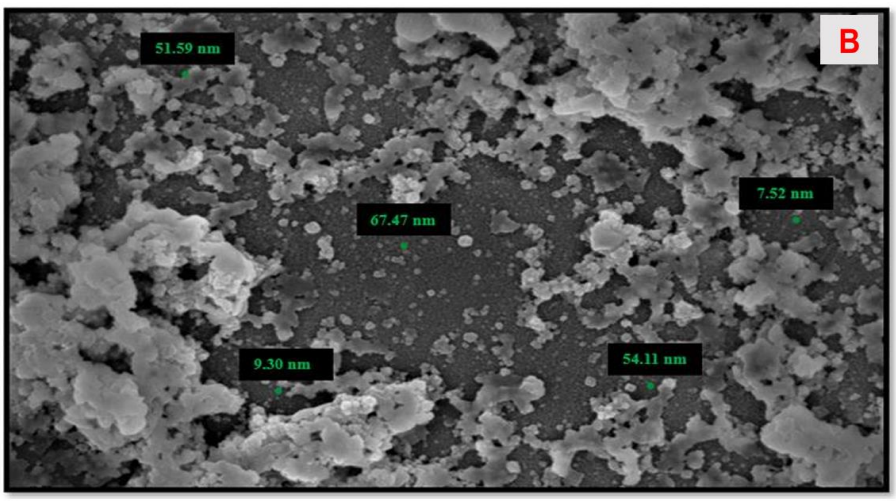


Fig. 3. FTIR spectra of copper nanoparticles synthesized from the leaf extracts of *Lawsonia inermis, Eucalyptus globulus, Psidium guajava,* and *Azadirachta indica*





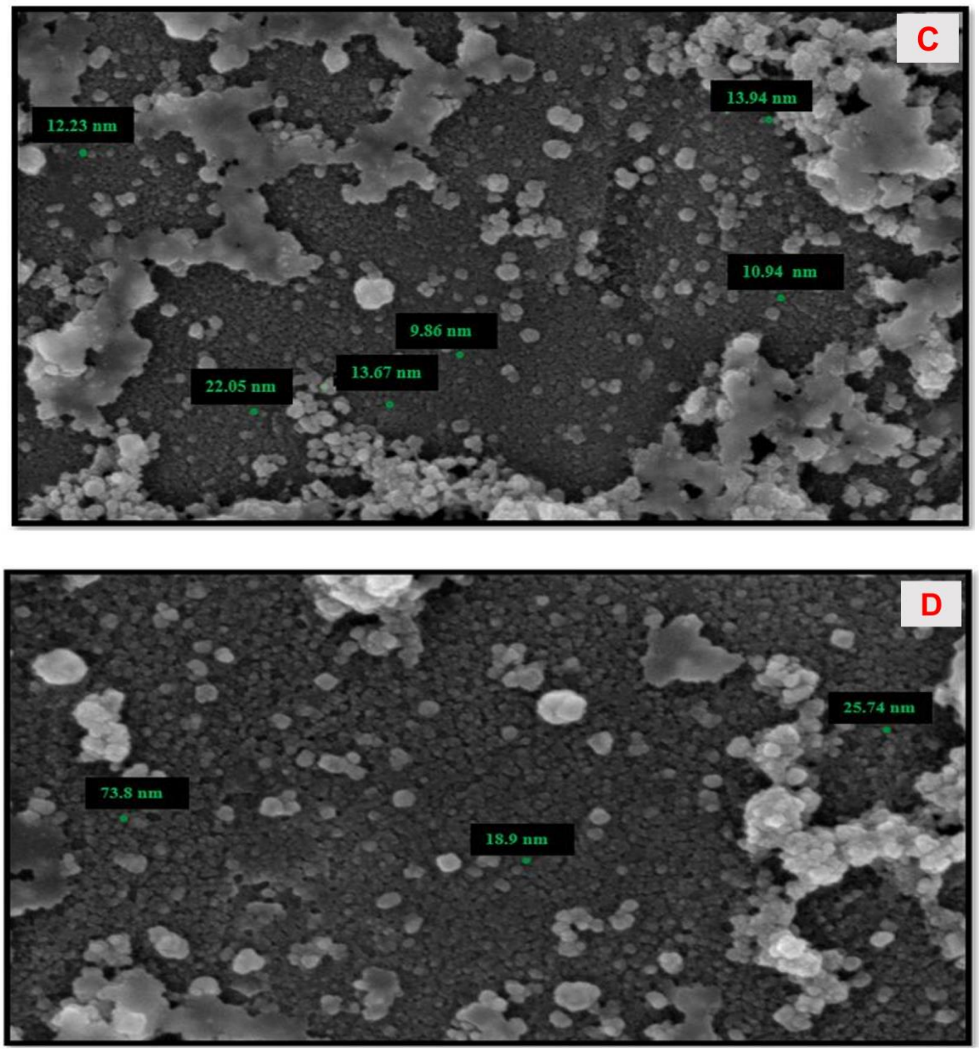


Fig. 4. The images of copper nanoparticles synthesized from the leaf extracts of *Lawsonia inermis (A), Eucalyptus globulus (B), Azadirachta indica (C), and Psidium guajava* (D) resulted from field emission scanning electron microscopy

Table 2. The particle size of copper nanoparticles (CuNPs) synthesized from the leaf extracts of *Lawsonia inermis*, *Eucalyptus globulus*, *Psidium guajava,* and *Azadirachta indica* resulted from field emission scanning electron microscopy

Sr. No.	Type of CuNPs	Size ranges (nm)
1.	*Lawsonia inermis* CuNPs	8.20-15.94
2.	*Eucalyptus globulus* CuNPs	7.52-67.47
3.	*Psidium guajava* CuNPs	18.90-73.80
4.	*Azadirachta indica* CuNPs	9.86-22.05

4. CONCLUSION

The CuNPs synthesized from the leaf extracts of *L. inermis*, *E. globulus*, *A. indica,* and *P. guajava* showed colour change with characteristic surface plasmon resonance bands. The plasmon resonance bands of CuNPs synthesized using the leaf extracts of *L. inermis*, *P. guajava,* and *A. indica* peaked at a shorter wavelength (300nm) than *E. globulus* CuNPs (400nm). The CuNPs synthesized from *L. inermis*, *E. globulus*, and *P. guajava* exhibited brown colour while those synthesized from *A. indica* leaf extracts showed green colour. Eleven bands were present in the CuNPs synthesized from the leaf extracts of *L. inermis* and *A. indica* while ten bands were in the

E. globulus and *P. guajava* CuNPs. The *L. inermis*, *E. globulus*, and *A. indica* CuNPs were spherical, except *P. guajava* CuNPs which were flaky in shape. The particle size of *L. inermis* CuNPs and *A. indica* CuNPs varied narrowly as compared to those synthesized from *E. globulus* and *P. guajava*.

ACKNOWLEDGEMENT

We thank the Professor and head of the Department of Plant Pathology, College of Agriculture, Latur, Maharashtra, India for providing research facilities and ICAR fellowship to the first author.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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