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Antibacterial Potential of Copper Oxide Nanoparticles Synthesized from Leaf Extract of *Terminalia muelleri* Benth

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Abstract: The present communication deals with phyto-chemical analysis and development of nano-materials from the leaf extract of *Terminaliamuelleri* along with characterization and antibacterial activity of the developed nanomaterials. The leaves of *T. muelleri* are rich in flavonoids, steroids, phenolics, tannins and terpenoids. The maximum UV-VIS absorption spectra at 260nm showed green synthesis of copper oxide nanoparticles (CuONPs). The flavonoids, tannins, and other phenolic compounds present in the *T. muelleri* leaf extract reduced copper ions into CuONPs. The possible functional groups present in the plant extract were confirmed by the spectral analysis of FT-IR studies. Similarly, the formation of crystalline structure of the powdered CuONPs was confirmed by XRD. The SEM-EDAX results showed the particles were spherical shaped and mono-dispersed and the synthesized NPs are pure elemental in nature. The 1000ppm of synthesized CuONPs showed significant antibacterial activity against both B. cereus and E. coli. Hence the synthesized CuONPs have potential antibacterial activity and could be further used for the development of nano-drugs against *Bacillus cereus* and *Escherichia coli*.

Key words: Antibacterial, Characterization, Green synthesis, Nanoparticles, Copper Oxide

Introduction

The family Combretaceae is an economically important family and different species of plants belonging to this family are used as traditional medicine systems of different countries all over the world (Roxburgh, 1811). The members of Combretaceae family comprises of about 200 species growing in the tropical regions around the globe (Gangopadhyay and Chakrabarty, 1997; Chakrabarty et al., 2019). The genus *Terminalia* (L.) ismedicinally important and the second-largest genus of the family Combretaceae. Different parts of *Terminalia* have been used in ancient traditional, cultural,indigenous and folk medicines in South East Asia, Egypt, Africa, India and Pakistan for the treatment of various problems, including gastrointestinal disorders, microbial infections, cough, cold, gastric ulcers, skin problems and sore throats as it contains diverse amount of secondary metabolites including terpenoids, flavonoids, catechin, tannins, and phenolic compounds (Eloff et al., 2008). Many biological activities have been reported by different researchers for *Terminalia* including antimicrobial, antioxidant, antipyretic, hepatoprotective, antihyperlipidemic, antidiabetic, anti-inflammatory and cytotoxic activities (Zhang, 2019).

In traditional Ayurvedic medicinal system of India, *Terminalia* (Behda, Harad, Arjuna) has been used widely to balance the three doshas that is kapha, pitta and vata. Traditionally species of *Terminalia* has been used for many disorders like, asthma, cardiovascular problems, cough, bile duct and digestive-related disorders, scorpion stings, and various types of poisoning. The bark of *T.arjuna* has been used in India for more than 3000 years, primarily as a heart remedy. The plant is used as a street tree in numerous cities, including India, Australia, China and Singapore. The leaves of this genus contain many bioactive metabolites with biological activities like antibacterial and antioxidant properties. Gallic acid and ellagic acid are the main active chemical compounds present in fruits (Fahmyet al., 2015).

With the advancement of science and a run for synthesis of nanomaterials, as it is ultrafine 1-100nanometers (nm) in size. But the chemical synthesis of nanomaterial cost effective and hazardous to environment. Hence the production of nanoparticles (NPs) by using biological techniques gained attention as it is low-cost and environmentally safer process. To date, organisms including bacteria, fungi, yeast and plants have been used to produce NPs (Salem et al., 2023). Researchers have been synthesizing nano-materials using biological entities to reduce negative impact on nature (Salem, 2023). A new, rapid and recently developing eco-friendly science is the synthesis of plant-based nano-materials. As survey of literature revealed that many species of the genus *Terminalia*are used for the biosynthesis of metal NPs (El-Rafie and Hamed, 2014).

The present work is focused on phytochemical screening of *T.muelleri* for secondary metabolites along with the development and characterization of copper oxide nanoparticles (CuONPs) from the leaf extract of *T. muelleri* and their anti-microbial activity against different bacteria.

Materials and Methods

Plant material

The plant material was collected from the Chaudhary Charan Singh University, Meerut, Uttar Pradesh, Indiaand identified as *Terminaliamuelleri*Benth with the help of "Flora of Upper Gangeticplain, and of the adjacent Siwalik and sub-Himalayan tracts" by J. F. Duthie, (1903), Forest Flora of the Chakrata, Dehra Dun, and Saharanpur Forest Divisions, Uttar Pradesh, India by Kanjilal (1969), Flora of British India by Hooker (1885).

The leaves of *T. muelleri* were washed thoroughly with mild detergent (Titron-X) to remove any impurities and dried at 60°C for 10 days to remove moisture. The dried leaves were ground into a fine powder and passed through 0.2mm sieve to remove large particles and stored for further analysis.

Phytochemical screening of plant material

Phytochemical screening of the powdered leaves was done for the presence of different secondary metabolites. The presence of flavonoid compound was tested by Shinoda test (Shinoda, 1928) and alkaline reagent test (Garg and Garg, 2019); Juglone test A was done for the presence Naphthoquinone (Gibbs, 1974); Ehrlich test B for Leuco-anthocyanins (Gibbs, 1974); Bontrager's test for Anthraquinones (Harborne, 1973); Liebermann-Burchard test for steroids (Nath et al., 1946); Foam test for saponins (Gibbs, 1974); Killer-Killani test for cardiac Glycosides (Gibbs, 1974); Ferric chloride test (Soloway and Wilen,1952), Gelatin test (Harborne, 1973) and Lead acetate tests (Harborne, 1973) for presence of phenolic compounds; test for catecholic tannins (Garg and Garg, 2019) and Ninhydrin test for the presence of terpenoids (Kumar et al., 2017).

Preparation of plant extractfor the synthesis of NPs

For the preparation of leaf extract, 1gm powdered plant material was dissolved in 100ml of distilled water and heated on a hot-plate magnetic stirrer at 80°C under continuous stirring at 1000 rpm for 30 minutes. The color of the solution was changed to dark brown. The plant extract was allowed to cool at room temperature and the extract was filtered with Whatman no.1 filter paper after centrifugation at 5000 rpm for 20 minutes and stored at 4°C for further characterization.

Green synthesis of copper nanoparticles (CuONPs)

For the green synthesis of nanoparticles 50ml of plant extract were mixed in 500 ml of freshly prepared 0.2M $CuSO_4$ solution to make final volume of 10% solution. The color of the mixture was turned to green after the addition of the leaf extract. The mixture was allowed to incubate for 24hrs at room temperature. The change of color from green to dark brown indicated the formation of CuONPs. The CuONPs were separated from the reaction mixture by the process of centrifugation. The obtained nanoparticles were washed with distilled water, and then dried and stored for further analysis

Optical analysis by of CuNP by UV-visible spectrophotometer

The optical properties of synthesized nanoparticles were characterized by UV-spectrophotometer (Shimadzu 2600i) in the range from 200 to 700nm for the absorbance spectra.

FTIR-analysis

The presence of functional groups present in the plant extract was carried out by FTIR (Shimadzu, IR Affinity-1S) to find out possible functional groups acting as reducing agents for synthesis of CuONPs.

XRD (X-ray diffraction) analysis

The crystalline analysis of the NPs was recorded in between 10° to 80° by XRD (Rigaku Smart lab coupled with HyPix-3000 high energy resolution multidimensional goniiometer detector with Cu Kalphaλ=1.5418A radiation) for confirming the formation of crystalline structure.

SEM-EDAX (Scanning electron microscopy) analysis

Scanning electron microscopy- Energy dispersive X-ray analysis (SEM-EDAX) (Carl-Zeiss-Ultra Plus Field- Emission Scanning Electron Microscope) was used to analyze the surface morphology and for the analysis of elemental composition of synthesized CuNPs.

Microbes and their growth conditions

Pure bacterial cultures of *Bacillus cereus* and *Escherichia coli*are available at Algal Biotech Laboratory, Department of Botany, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India. The bacterial strains were cultured in Muller Hington Broth (MHB, Merck) in rotary incubator at 1000rpm at 37°C for 24 hrs for uniform growth of the culture organism (Gonelimaliet al., 2018).

Antibacterial assay of CuONPs

Antibacterial assay was done by the well diffusion method (Tailor et al., 2020). A concentration dose of 1000ppm, 500ppm, 250ppm, and 125ppm of CuNPs in double distilled water was prepared and 50µl of different concentrations of CuNPs was inoculated in each well. Tetracycline was used as positive control. The antimicrobial activity was measured by the zone of inhibition in diameter (mm).

Results and Discussion

The results of phytochemical screening were recorded as positive and negative on the basis of reaction color and formation of precipitate (Table-1).

Table-1: The results for phytochemical screening of powdered leaves of T. muelleri

S. N.		Phytochemical Assay	Extract	Result
1.	Flavonoid tests			
	a)	Shinoda test	Ethanol extract	Positive
	b)	Alkaline test	Aqueous extract	Positive
2.	Juglone test for Naphthoquinone		Aqueous extract	Negative
3.	Ehrlich B test for Leuco-anthocyanin		Aqueous extract	Negative
4.	Bontrager's test for Anthraquinone		Aqueous extract	Negative
5.	Liebermann-Burchard test for Steroids		Aqueous extract	Positive
6.	Foam test for Saponins		Aqueous extract	Negative
7.	Killer-killani test for cardiac Glycosides		Aqueous extract	Negative
8.	Phenolics			
	a)	Ferric chloride test	Aqueous extract	Negative
	b)	Gelatin test	Methanolic extract	Positive
	c)	Lead acetate test	Methanolic extract	Negative
9.	Tannin		Aqueous extract	Positive
10.	Terpenoids		Aqueous extract	Positive

Optical analysis by UV-visible spectrophotometer

The change of color from greenish to dark brown in reaction mixture confirmed the formation of CuONPs (Figure-1A-D). The optical properties of synthesized NPs were measured in the range from 200 to 800nm. The detailed result optical analysis of synthesized CuONPs is given in Figure-2. The maximum absorption was recorded at 260nm. The absorption spectra obtained are similar to the absorption spectra obtained by Turakhia, et al. (2020) and Abdullah, et al. (2022) indicating proper synthesis of the CuONPs.

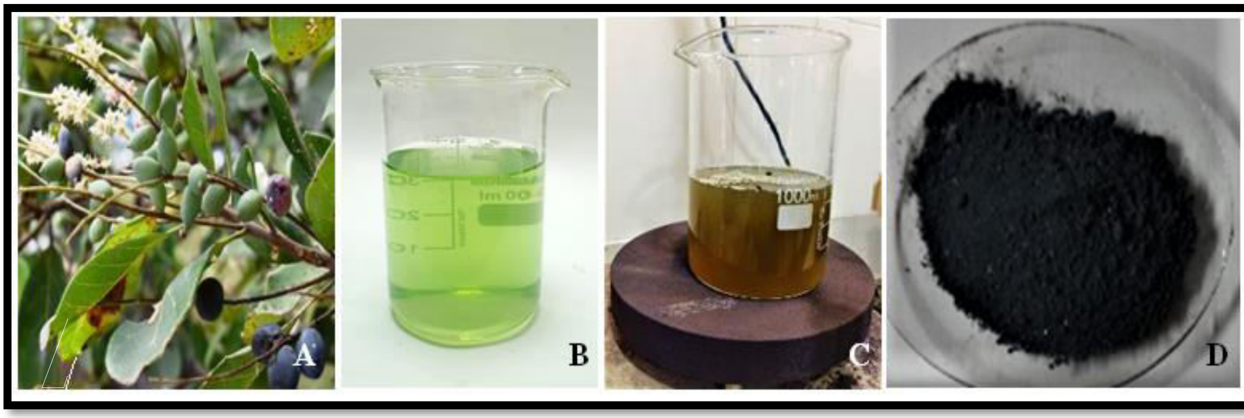

Figure-1(A-D): Figures describing (A) *Terminaliamuelleri*, (B) Plant extract in distilled water, (C) Change in colour of plant extract due to synthesis of CuONPs, (D) Oven dried CuONPs

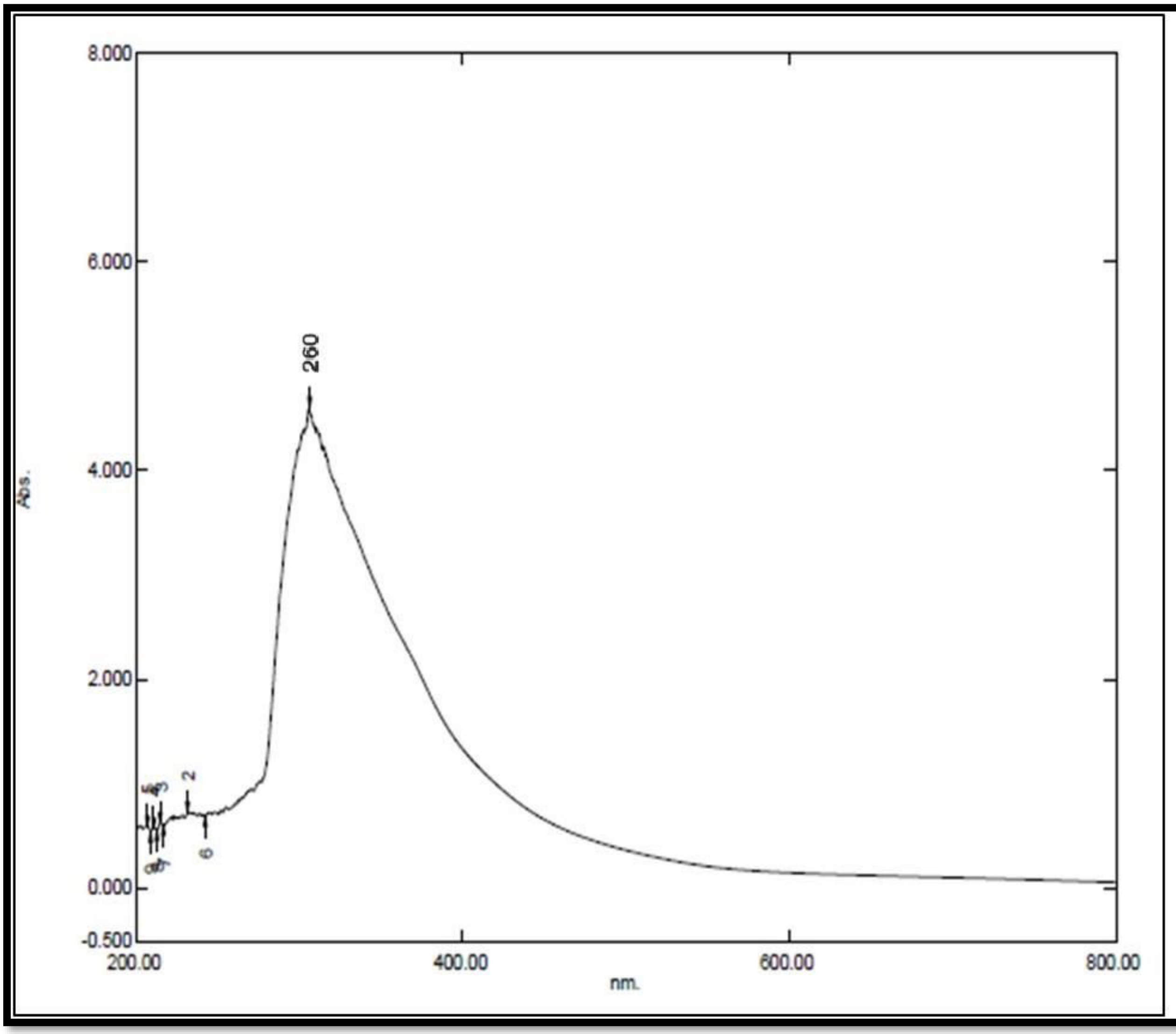

Figure-2:UV-VIS analysis of synthesized CuONPs

FTIR-analysis

The peaks for synthesized CuONPs were observed at 2920, 2372, 1039, 545,520 and 530cm-1. The peaks at 3422, 2920, 2372, 1627 and 1036 corresponds to O-H stretching, alkaline stretching, C=C and C-H vibrations respectively (Renugaet al., 2020).The strong peak at 530 and 450 cm^{-1} corresponds to the deformation vibration of copper oxide (Cu-O) (Zhang, et al., 2009; Radhakrishnan&Beena, 2014; Jońca et at., 2017; Dehaj&Mohiabadi, 2019). The frequency assignment of FTIR spectra for synthesized CuONPs is given in Table-2. FTIR analysis results also show the possible functional groups present in the plant extract that acts as reducing agents for synthesis of CuONPs. The FTIR spectra for CuONPs in given in Figure-3.

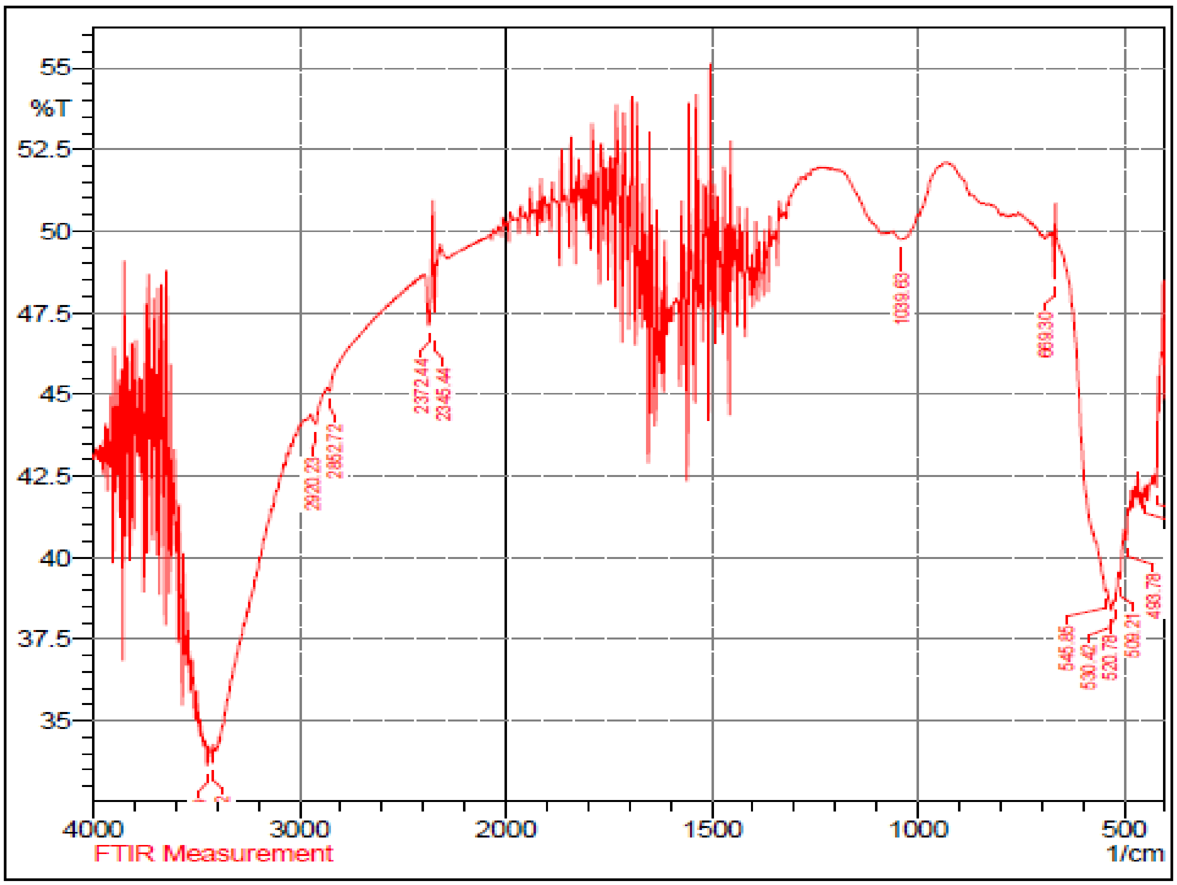

Figure-3:FTIR analysis of synthesized CuONPs

Table-2: Frequency assignment of FTIR spectra for synthesized CuO NPs.

Sl. No.	Frequency (cm-1)	Functional group
1.	2920	O-H stretching
2.	2852	O-H stretching
4.	1039	CH deformation, C-O, C-C stretching
5.	545	vibration of Cu-O
6.	530	vibration of Cu-O

XRD (X-Ray diffraction) analysis

The positions of the 2Θ values at 32.29°, 35.40°, 38.48°, 48.65°, 53.32°, 58.09°, 61.33° and 66.00° are indexed at (110), (002), (111), (202), (020), (202), (113) and (313) planes, which are in good agreement with those of powdered CuO obtained from the international centre of Diffraction data card (JCPDS-45-0937) confirming the formation of crystalline structure similar to the results obtained by Ghidan, et al. (2016). A detailed result on XRD pattern of synthesized CuONPs is given in Figure-4.

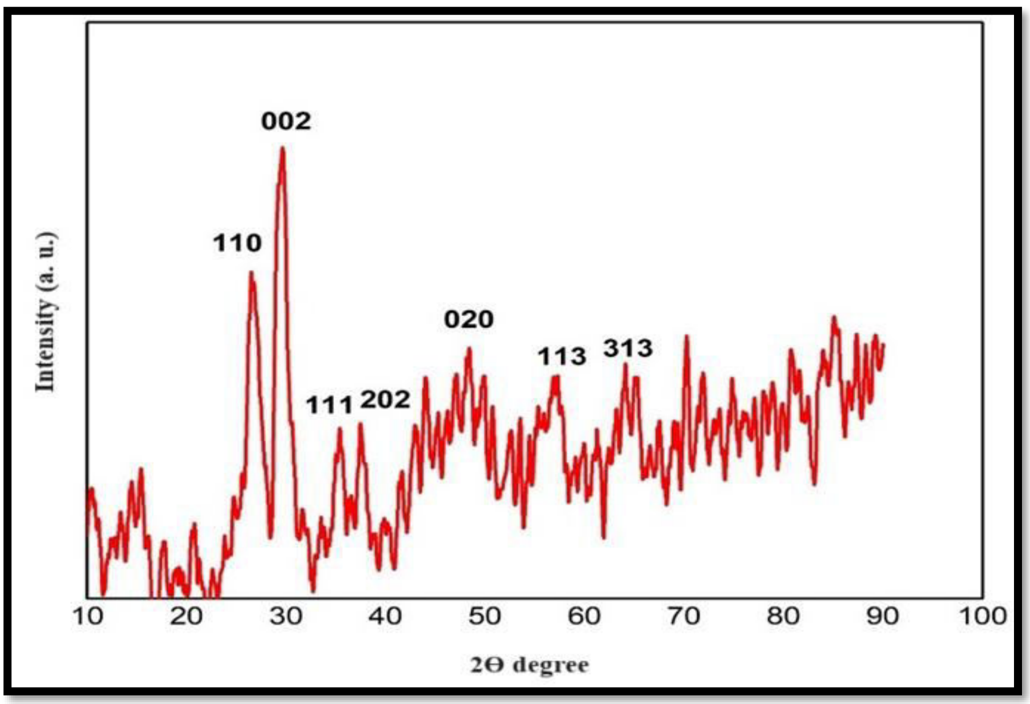

Figure-4: XRD pattern of synthesized CuONPs

SEM-EDAX analysis

The synthesized CuONPs are in spherical shape with aggregation. The aggregation of particles is due to functional groups of secondary metabolites present in the plant extract which is used as a reducing agent for the formation of metal oxide while the purity and elemental analysis is carried out by EDAX showing the highest peaks for copper showing 76.73% and oxygen 23.23%. Detailed surface morphology of CuONPs and purity of the synthesized CuONPs is given in Figure-5.

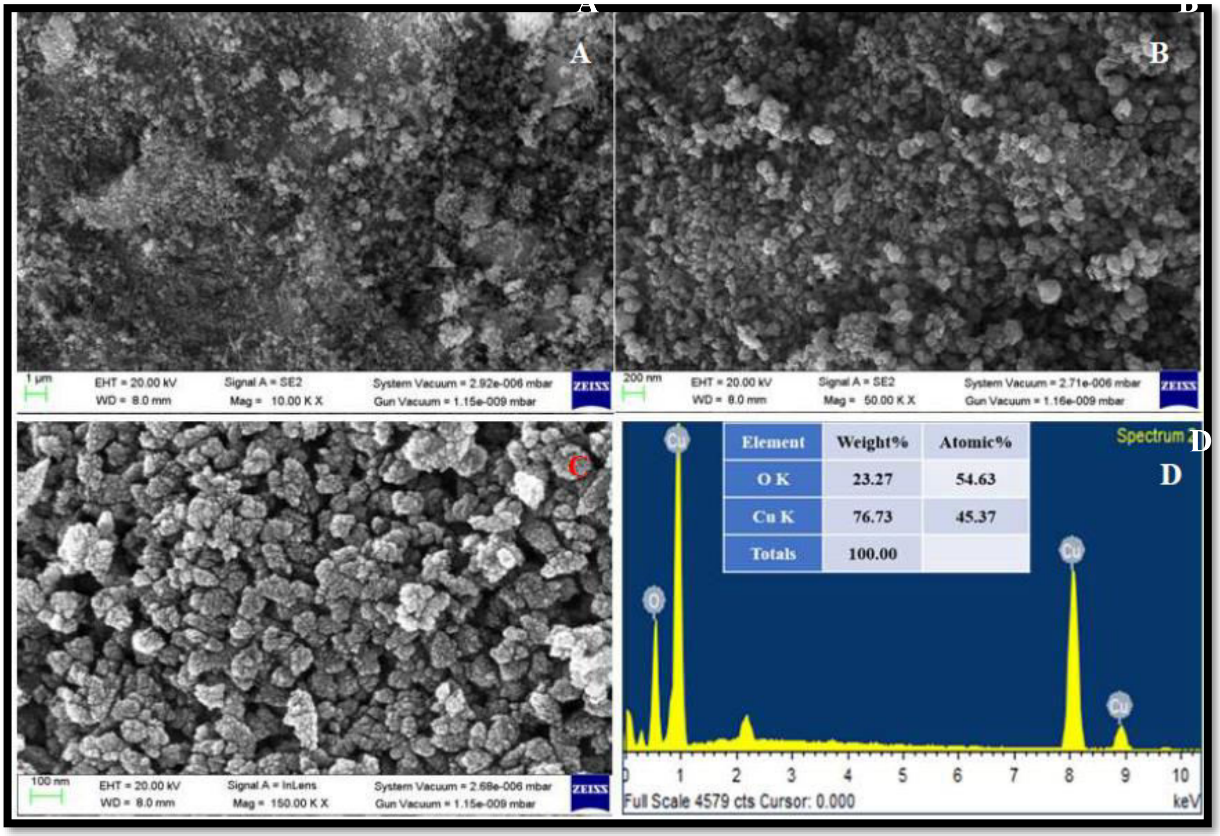

Figure-5(A-D):(A) 1µm SEM micro-pictogram, (B) 200nm SEM micro-pictogram(C) 100nm SEM micro-pictogram and (D) EDAX images of synthesized CuONPs.

Antibacterial activity

The visualization of clear inhibition zone against different concentrations of the tested nanoparticles around the wells suggests that the green synthesized CuONPs shows antibacterial activity which is able to inhibit the growth of gram-positive bacteria *B. cereus*and gram negative bacteria *E. coli*. The antibacterial activity on application of 500ppm and 1000ppm CuONPs is almost similar indicating that >500ppm CuONPs could inhibit the growth of *E. coli*. Similarly, in case of B. cereus maximum zone of inhibition was observed at 1000ppm, while a concentrationof 500ppm CuONP could inhibit almost similar to that of 1000ppm. The zone of inhibition against *B. cereus* and *E. coli* respectively is given in Figure-6(A-B). Detailed results on well diffusion of the different concentrations of CuONPs are summarized in Fig.-7.

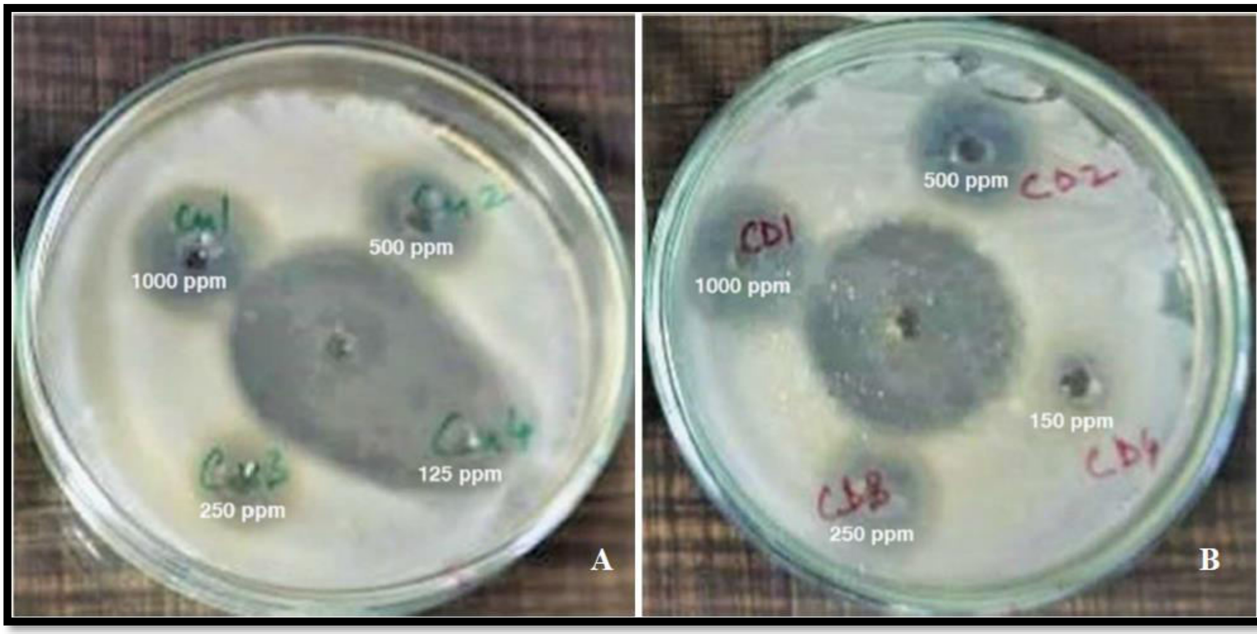

Figure-6(A-B): Zone of inhibition of CuO NPs against (A) *Bacillus cereus*, (B) *Escherichia coli*.

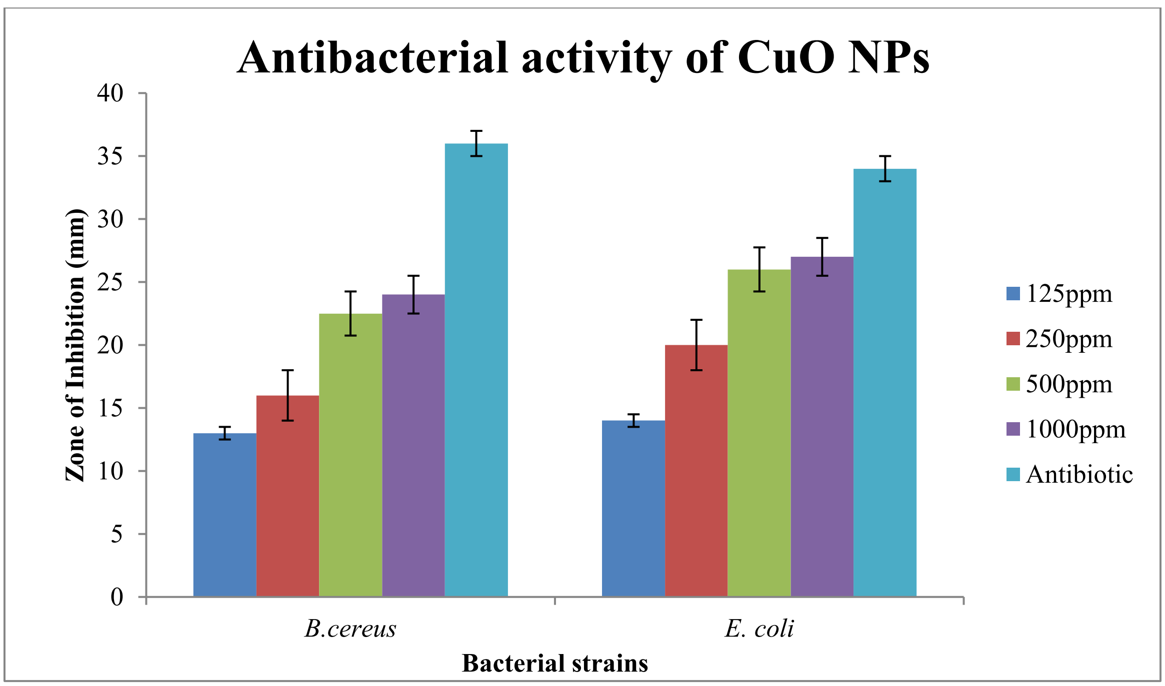

Figure-7: Antibacterial activity of CuONPs against *B. cereus* and *E. coli*.

Conclusion

From the results obtained, it could be concluded that the leaf extract of *T. muelleri* could be a potential raw material for the development of nano-materials. Confirmation for the development of CuONPs was indicated by a change in colour of the leaf extract and by the absorption maxima at 260nm. The flavonoids, tannins and other phenolic compounds present in the *T. muelleri*leaf extract reduced copper ions into CuONPs. The possible functional groups present in the plant extract were confirmed by the spectral analysis of FT-IR studies. Similarly the formation of crystalline structure of the powdered CuONPs was confirmed by XRD. The SEM results concluded that the particles were spherical shaped and mono-dispersed. The pure elemental nature of synthesized NPs was examined by EDAX studies. The synthesized CuNPs showed significant antibacterial activity against the both bacteria B. cereus and E. coli. It can be concluded that CuONPs successfully synthesized by eco-friendly green method and used as antimicrobial agents.

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