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Antibacterial Activity and Phytochemical Analysis of Biosynthesized Copper Nanoparticles Produced by *Nyctanthes arbortristis* Leaves Extract

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Abstract

Nyctanthes arbortristis, also known as Night jasmine or Parijaat, is a most common medicinal plant in India and its neighbouring countries. In this work, *Nyctanthes* leaves extract was used in the green synthesized copper nanoparticles. An examination of the *Nyctanthes* leaves extract's phytochemistry reveals the presence of substances including steroids, flavonoids, and polyphenols, tannins, proteins and carbohydrate compound. The secondary metabolites were confirmed by specific test. Scanning electron microscopy SEM, EDX, XRD, TEM and UV-Spectra were used to analyze the biosynthesized copper nanoparticles. Against the gram-negative *Pseudomonas aeruginosa* bacteria, biosynthesized copper nanoparticles demonstrated antibacterial properties. The results of that study show greater value of zone inhibition was found to be 12 mm at 200 µg/mL of CuNPs concentration of *Nyctanthes arbortristis*. The study concluded that the *Nyctanthes arbortristis* plant leaves extract produced copper nanoparticles that have antibacterial activity. Antibacterial sensitivity was proof against *Pseudomonas* bacteria. Preliminary phytochemical studies show the presence of secondary metabolites such as alkaloids, Protein, Carbohydrates, phenols and terpenoids.

Key words: *Nyctanthes arbortristis*, Antibacterial, Biosynthesis, CuNPs, *Nyctanthes* leaves

Nanobiotechnology and nanoscience is an emerging field which aims to exploit the potential of a growing number of nanomaterials. It combines Fundamental sciences like physics, chemistry, and electronics are combined with material science approaches to create molecules with uncommon and distinctive features [1-2]. Nanobiotechnology and nanoscience is an emerging field which aims to exploit the potential of a growing number of nanomaterials. It combines fundamental sciences like physics, chemistry, and electronics are combined with material science approaches to create molecules with uncommon and distinctive features [1-2]. Because copper is profitable and has a long history of usage as a powerful antibacterial agent, it can be used to make a special class of biocidal compounds. These investigations support the utilization of copper nanoparticles is in the creation of medicines and pharmaceuticals [3]. Copper compounds were employed in a variety of forms during the ancient times [4]. Common techniques such metal vapour synthesis, the bursting cable, vacuum evaporation, generate crystal nuclei and transparent emulsion were used to handle this noble metal copper nanoparticle [5-8]. Green or environmentally friendly products, such as plants, enzymes, microorganisms, etc., not only disregard the dangers of toxic compounds but also need little effort and little money. These methods demand costly materials, tremendous pressure, radiation, and hazardous chemicals, such as those used in nuclear power plants, for example [9]. To create metal nanoparticles for application in medicine and other industries, copper nanoparticles are being created utilizing a variety of plant extracts, such as tea leaf. This is due to the fact that they are readily available, safe for the environment, and the main driver of less laborious purification stages [10-13]. According to several studies, copper nanoparticles that are bioactive allow for greater environmental mobility as well as close contact and entry into bacterial membranes [14], *Murraya Koenigii* leaves [15], *Eclipta prostrata* leaves [16], *tomato* [17], peel extract [18] and *Aegle marmelos* leaf [19], etc.

The sacred Oleaceae species *Nyctanthes arbor tristis*, commonly known as night jasmine (Parijata), was regarded as one of the five the gratification of a desire especially symbolically plants in Hindu's culture mythology. The plant's phytochemical analysis indicated the presence of volatile oils, ascorbic acid, and flavonoids, phenols, glycosides, mannitol, and acid [20-21]. The extract from the leaves has several pharmacological properties that have been demonstrated, including antibacterial, analgesic, anti-inflammatory, anti-diabetic, anti-arthritic, antioxidant, hepatoprotective, and antispasmodic actions [22-23]. Numerous uses for the created nanomaterials have been reported, including the development of drug delivery systems, fuel cells, photocatalysts, Antibiotics, chemotherapy, immunotherapy, antifungals, antiparasitics, antioxidants, enzymes, biosensors, and theragnostic.

There are still several issues with synthesis carried out by plants approach which ought to resolve, for example, the

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intricacy and variety bio-reduction of phytochemicals in plant systems processes, similitude intensify, repeatability, easily available material to everyone and balanced consequences. Copper has attracted a lot of interest from scientists studying nanotechnology, particularly those working in the field of nanomaterials. This is due to copper's (Cu) lower cost, higher availability, and greater conductivity when compared to silver (Ag) and gold (Au). In light of this, several Cu nanomaterials have been created, and there is also a good source of antioxidant.

MATERIALS AND METHODS

Plant collection

Nyctanthes arbortristis plant were collected from different places such as agriculture field of Firozabad and Shikohabad Uttar Pradesh in the month of April-June 2021.

Fig 1 *Nyctanthes arbortristis* plant

Fig 2 *Nyctanthes* leaves

Preparation of plant extracts

The collected leaves were air dried for in order to prepare them for further research, they were dried for 20-30 days, ground into a fine powder, and then stored.

Using the Soxhlet extraction method, the 20gm powder of leaves was extracted with ethanol for 48 hours. Filter paper from Whatman was used to filter the extracts. By using column chromatography, the filtrates were made clean. The clean extracts were then heated to temperatures between 35 and 40 degrees Celsius at room temperature and coated with porous silver foil in a beaker. The dried extracts were then placed in an airtight container for analysis.

Fig 3 Extraction with Soxhlet apparatus

Fig 4 Phytochemical studies of *Nyctanthes arbortristis*

Preliminary phytochemical screening

The prepared extract was ready to use in identification of secondary metabolites by specific test such as for Alkaloids- Wagner's test was conducted. For carbohydrate- Molisch test was conducted. For Phenol- ferric chloride test was conducted. For Protein – Xanthoproteic test was conducted. For terpenoids Salkowski test was conducted.

Plant mediated CuNPs preparation

For the difficult synthesis of CuNPs, 60 ml of a 0.1 mM copper sulphate pentahydrate solution and 20 ml of *Nyctanthes arbortristis* plant leaf extract were mixed continuously at a temperature of 60–70°C while being stirred. Visual inspection revealed a colour shift in the solution, which supported the creation of Cu nanoparticles. Nanoparticle were separated by centrifuge apparatus at 5000-8000rpm. Settle down nanoparticles were dried in oven at 80-90°C for some time.

Fig 5 Confirmation by colour change reaction of CuNPs

Techniques for characterizing synthesized nanoparticles

Various approaches were used to characterized biosynthesized mediated nanoparticles, such as ultraviolet-visible (UV-Vis) spectroscopy examination to identify nano copper. Field Emission Scanning Electron Microscope (FE-SEM) analysis of shape and size and X-ray diffraction analysis of the crystal structure of the produced CuNPs (XRD). The chemical states and elemental composition of the produced copper nanoparticles were described by EDX. The shape of CuNPs were examined by (TEM) Transmission Electron Microscopy.

Antibacterial activity of copper nanoparticle

For the purpose of evaluating the antibacterial activity of biologically produced CuNPs [24], the disc diffusion method was used. Overnight, the bacterial strain was raised in nutrient broth at 28°C. A thick grass was created on the Nutrient agar plate using the *Pseudomonas aeuriginosa* inoculum (100 µl). Then, 30 µl of the CuNPs sample were impregnated into a paper disc. CuNPs were diluted in concentrations of 200, 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL. The clear zone of inhibition that surrounded the discs was measured in mm (diameter).

RESULTS AND DISCUSSION

Because of their distinctive optical characteristics, nanoscale materials are generating a lot of interest. During the biogenesis process, nanoparticles displayed a wide range of colours. A plant extract's ability to react with $CuSO_4$. $5H_2O$ and form copper nanoparticles was principally determined by the reaction mixture's colour, which changed from greenish blue to brown in under one hour. After 24 hours, the colour shift halted and precipitation was seen, indicating that the process of creating the nanoparticles was complete. Similar colour changes were seen by Ashtaputrey *et al.* [25] during the production of CuNPs at various incubation periods.

Results of preliminary phytochemical studies

Table 1 Shows the preliminary phytochemical analysis

	Alkaloids	Phytosterols	Flavonoids	Carbohydrate	Phenols	Proteins	Steroids	Terpenoids
MLE	+	+	-	+	+	+	+	+

+ Present, - Absent

Evaluation of phytochemicals shows the occupancy of some secondary metabolites such as alkaloids, phytosterols, carbohydrate, phenols, proteins, steroids and terpenoids. Flavonoids was not present in *Nyctanthes* leaves extract.

Analysis of CuNPs using UV spectroscopy

The green manufactured copper nanoparticles' UV-absorption spectra were captured at a wavelength other than the 200-800 nm range depicted in (Fig 4). *Nyctanthes arbortristis* leaf extract used as a capping and reducing agent, and copper sulphate was used to create the copper nanoparticles, which have an absorbance peak at 405 nm. The peak may be attributed to CuNPs made synthetically using plant extracts [26].

SEM analysis of copper nanoparticle

(Fig 5) displays pictures of CuNPs created using *Nyctanthes arbortristis* leaf extract. By using this environmentally friendly process, the plane, shape and size of the copper nanoparticles revealed a nearly monodisperse distribution of particle sizes. The nano copper's typical particle size was found to be around 15 nm. It primarily displays rectangular CuNPs as well as a variety of aggregates, manufactured nanoparticles and occasionally nanoparticles with an ambiguous shape.

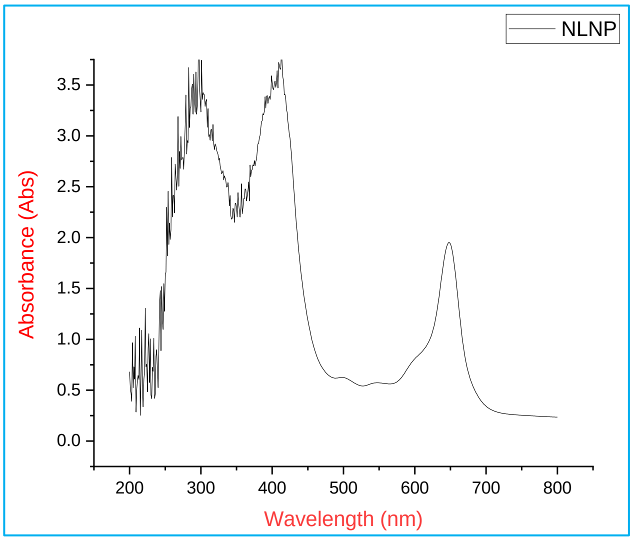

Fig 6 UV-Graph of *Nyctanthes* Leaves CuNPs

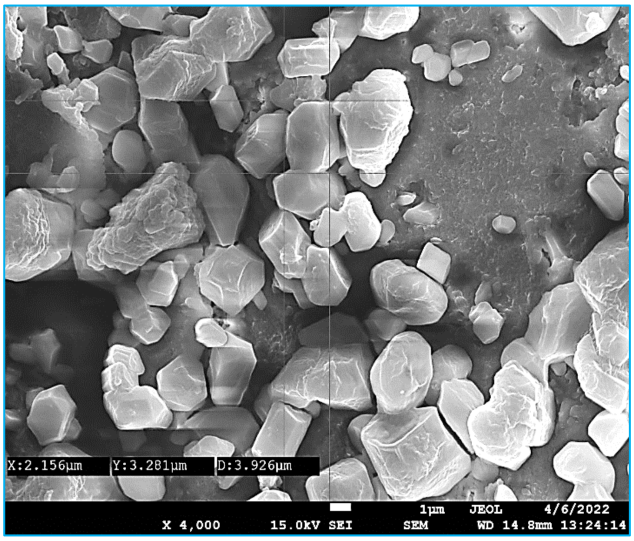

Fig 7 SEM image of NLNP

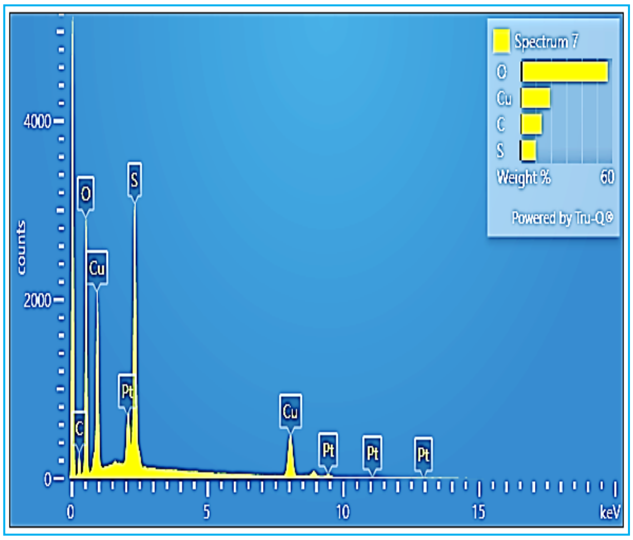

Fig 8 EDX image shows the presence of copper nanoparticle

Table 2 Stability and compositional characterization of the particles of copper nanoparticles

Element	Weight %	Oxide	Oxide %
C	13.60	CO_2	50.95
O	55.57		
S	9.29	SO_3	24.72
Cu	21.54	CuO	24.32
Total	100.00		100.00

Copper nanoparticles' XRD spectrum

The crystallinity of the nanoparticles is revealed by the XRD spectrum. By applying X-ray diffraction examination, it was determined that the nano copper created from *Nyctanthes arbortristis* leaf extract actually existed (Fig 7). For copper nanoparticles, two lattice planes, (111) and (200), were found at the reflection angles of De Bragg, which are 35.5 and 43.2, respectively. These distinctive peaks demonstrated that the crystalline nature of the produced copper nanoparticles. Here, there were a few unassigned peaks that could indicate less bio chemicals. Similar to this, Nasrollahzadeh *et al*. [27] observed that *Euphorbia esula* leaf extract (aqueous extract). produced crystalline copper nanoparticles. So, the newly synthesized result is pure crystalline content in nanoscale copper, according to XRD.

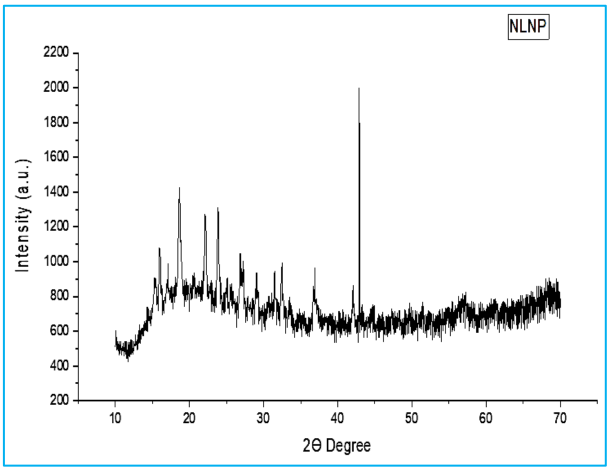

Fig 9 XRD Graph of *Nyctanthes arbortristis* leaves CuNPs

XRD analysis of the prepared sample of copper nanoparticle was done by X-ray Diffractometer Bruker D8 Advance, data was taken for the 2ϴ range of 10 to 70 degrees. Indexing process of powder diffraction pattern was done and Miller Indices (hkl) to each peak was assigned in first step. Diffractogram of the entire data is in (Fig 9).

Table 3 Particle size of copper nanoparticle of *Nyctanthes* leaves

Diffraction angle (Degree)	FWHM (Radians)	d-spacing (nm)	Diffraction plane (hkl)	Particle Size (nm)
43.62	0.0059	0.2073	(111)	20.32

The peak at 2ϴ value of 43.62 degree corresponding to (111) planes of copper were observed and compared with the standard powder diffraction card of JCPDS, copper file no. 04-0836, i.e., 43.297.

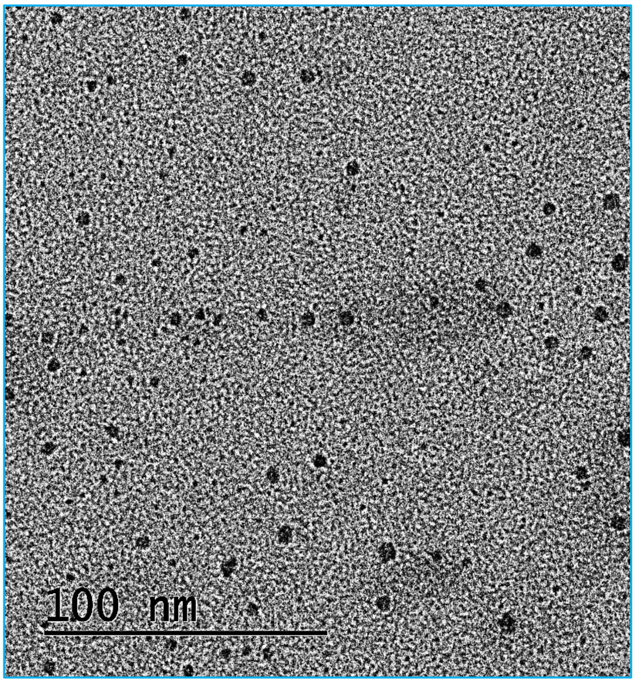

Fig 10 TEM image of CuNPs *Nyctanthes* leaves extract

TEM analysis of CuNPs leaves extract

TEM Model: Jeol JEM1400 (Jeol Ltd., Tokyo, Japan) TEM studies revealed the particle size of Plant extracted nanoparticles, at Central Drug Research Institute (CDRI) Lucknow. The particle size of *Nyctanthes* leaves nanoparticle is 1-12nm in range.

Copper nanoparticle's antibacterial activity

The results of current investigation, which are displayed in (Table 1, Fig 8-9), demonstrate CuNPs strong antibacterial activity. It has been noted that the information in (Table 1) provides the foundation for the antibacterial property of CuNPs (12mm in 200 µg/mL). Cefixime, an antibiotic, was utilized to manage *Pseudomonas aeruginosa* cells.

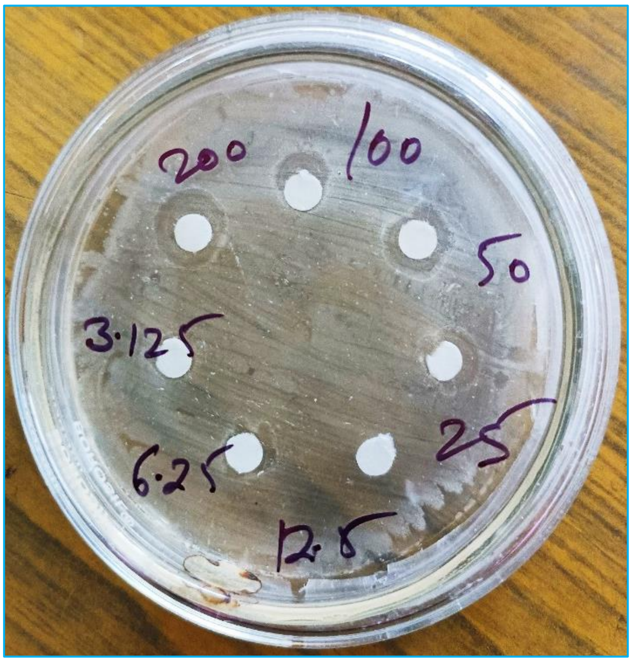

Fig 11 Antibacterial activity of cefixime (Reference compound-antibiotic)

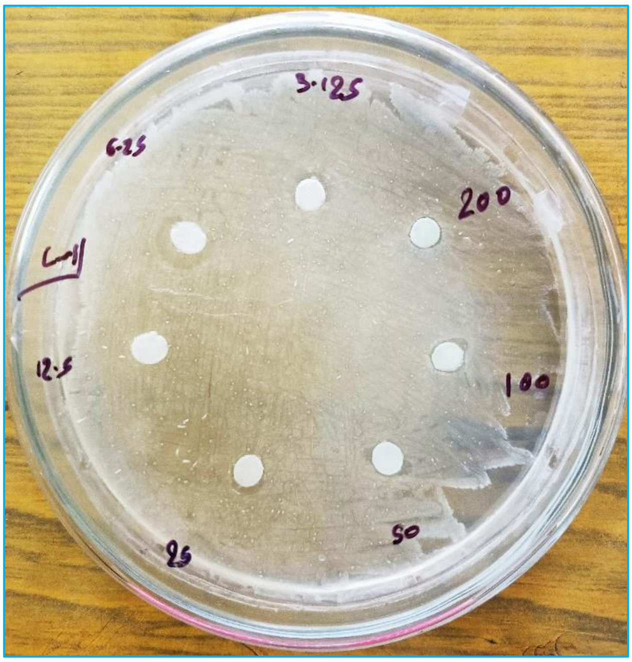

Fig 12 Zone of inhibition shows *Nyctanthes* CuNPs

Table 4 Maximum zone of inhibition

Compound / microorganism *Pseudomonas aeruginosa*	Inhibition zone (mm) (*Nyctanthes* leaves CuNPs)	Inhibition zone (mm) (Reference-cefixime)
Dilutions (µg/ml)		
200	12	18
100	9	16
50	8	15
25	6	13
12.5	4	12
6.25	-	-
3.125	-	-

This study found that green generated copper nanoparticles [28] based on *Nyctanthes arbortristis* leaf extract showed antibacterial action against cefixime antibiotic with *Pseudomonas* bacteria. Likewise, Lee *et al*. Subhankari *et al*. also synthesized CuNPs by green methods [29-30]. The location of the absorption peak at 405 nm in Ultraviolet spectra was used to originally establish the green production of copper nanoparticles. X-Ray diffraction reveals crystallized copper nanoparticles with a particle size of about 18 nm and a rectangular shape. EDX confirms the elemental composition of nanoparticle. Similarly, Bukhari *et al*. confirms with XRD presence of copper nanoparticle [31]. The location of the absorption band at 405 nm in UV spectra served as the primary proof that copper nanoparticles were produced using green methods. Crystalline nanostructured copper particles with a shape of oval, round, and some are rod shaped are visible, and the FE-SEM and XRD spectra confirm their rectangular shape [32-33].

CONCLUSION

The study found that the *Nyctanthes arbortristis* plant leaves extract produced copper nanoparticles that was confirmed by UV, FE-SEM, EDS, TEM and XRD techniques. CuNPs have antibacterial activity also that was confirmed by Disc Diffusion method. Antibacterial sensitivity was proof against *Pseudomonas* bacteria, the values of zone of inhibition were as same as the cefixime antibiotic tablet. Cefixime tablet was used as a reference compound. Preliminary phytochemical studies show being present some second-generation metabolites like alkaloids, protein, carbohydrates, phenols, and terpenoids.

List of abbreviations

CuNPs – Copper nanoparticles
NLNP – *Nyctanthes* leaves nanoparticles
NLE – *Nyctanthes* leaves extract

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