IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

# Exploration of Bio-synthesized Copper Oxide Nanoparticles Using *Ceropegia spiralis* wight. Tuber Extract by Antioxidant Activity and Biological Evaluations.

**Khaja peer Mulla[1], Suresh Pullani [2a], Shaheen Shaik [2b] and [*]Yasodamma Nimmanapalli[1]**

1 & 2b Department of Botany, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India.
2a Department of Botany, Bharathidasan University, Tiruchirappalli-620024, Tamil Nadu India.

**Abstract:**

In the present investigation, we report simple, robust and eco-friendly synthesis of copper oxide nanoparticles (CuONPs) using tuber extract of *Ceropegia spiralis*. Present study was aimed to synthesize copper oxide nanoparticles (CuONPs) by using *C. spiralis* tuber. Biosynthesized *C. spiralis* CuONPs was characterized by different spectroscopic techniques. UV–Vis spectrum showed the characteristic SPR peak 283 nm. EDX analysis revealed the presence of metallic copper oxide at 3 keV. XRD analysis clearly revealed that CuONPs are crystalline in nature with ECM structure. TEM analysis depicted the spherical morphology with 1.8 to 3.48 nm in size. DLS analysis showed that average hydrodynamic size and PDI value of *C.spiralis* CuONPs were found to be 59.7 nm and 0.466 respectively. Biosynthesized *C. spiralis* -CuONPs showed negative zeta potential value of – 16.0 mV. FTIR analysis revealed the participation of phenols and proteins in the bioreduction and stabilization of CuONPs. *C. spiralis* -CuONPs showed strong DPPH scavenging activity 66.01% 100µg/mL respectively. Antimicrobial studies of particles showed highest inhibitory activity against *Enterobacter aerogenes* (17 mm) and *Candida tropicalis* (14.25 mm) among bacterial and fungal strains, respectively. Further *C.spiralis*-CuONPs also showed effective cytotoxicity against cancer cells including HeLa (Human Cervix Adenocarcinoma) with maximum inhibition of 75.17% cell death and 24.83% cell viability respectively. This study paves a way to better understand antimicrobial and anticancer Therapeutic drug potentials of nanoparticles to design and analysis of pharmaceuticals by *in vivo* and *in vitro* approaches.

**Key Words:** Eco-friendly, Copper oxide nanoparticles, Phenols, bio-reduction, Cytotoxicity, HeLa.

## INTRODUCTION

Eco-friendly synthetic methods gain major research attention because it solves the problems associated with environmental pollution faced World-Wide. Utilisation of nontoxic solvents, closed reactors, 'green' techniques (biological methods, hydrothermal, ultrasound, magnetic, microwave, among others), and low temperatures are highly encouraged in order to attain a pollution free environment. Green-synthesised nanoparticles represent an innovative technique in the area of nanotechnology that is accomplished using plant extracts [1, 2], survey of literature proves that plant mediated nanoparticles synthesis stand for a better resource and also more suitable for a large-scale 'green synthesis' of the nanoparticles [3]. Henceforth, the biosynthesis of the nanoparticles is considered as a building block for the forthcoming generations by applying it in various medical fields. The synthesis of the metal and metal oxide nanoparticles requires secondary metabolite such as flavonoids, phenolic acid, terpenoids and alkaloids which are naturally present in various medicinal plants. These metabolites are involved in redox reactions to synthesize the environment friendly nano size particles [4]. In recent times, metal oxide nanoparticles produced by green synthesis served to be an appreciable method as it reduces the usage of toxic chemicals in biomedicine research areas [5-7]. Recently, the green synthesis of nano sized particles, flowers, wires, tubes were reported successfully. Functionally upgraded metal nanoparticles are excellent reserves for the multifaceted applications, especially in medicine, sensors, catalysis and energy studies, due to their remarkable changes in chemical, physical and optical properties [8]. Moreover, the usage of metal nanoparticles in various fields depends upon their chemical compositions, sizes, shapes, fine structure, and surface morphology [9]. The green synthesized CuONPs established an effective inhibitory activity against various pathogens viz. *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Escherichia coli*. [10]. the synthesis of CuONPs using different plants such as *Pterocarpus marsupium* [11], *Phaseolus vulgaris* [12], *Desmodium gangeticum* [13], and *Pterolobium hexapetalum* [14], have also been previously reported.

***C. spiralis*** Wight (Apocynaceae) is a slender, erect herb with depressed tubers, opposite leaves, sessile 10-20cm long narrowly linear, base and apex often curved and twisted at the tip. Flowers 3-5 cm long .greenish-purple, cymes, mostly solitary. Fruit of two slender follicular mericarp (Fig 1) [15]. Flowers peculiar with ornamental potential. it is endemic to Peninsular India [16].

The tuberous roots are edible [17], which contain starch, sugar, gum, albuminoids, fats, and crude fibers are valuable constituents in many traditional medicinal systems of India. *Ceropegia* species are storehouse of various valuable phytoconstituents that are routinely used in traditional Indian ayurvedic drugs for the treatment of gastric disorders, diarrhoea, dysentery, urinary tract ailments, etc [18]. Pharmacological importance of the genus *Ceropegia* is mainly due to the presence of pyridine alkaloid "cerpegin", which is potentially antipyretic, analgesic, local anesthetic, antiulcer, mast cell stabilizing, hepato-protective, tranquilizing and hypotensive [19].

Poor seed setting, low seed germination, scarcity of pollinators and indiscriminate exploitation of edible tubers of *Ceropegia spiralis* seems to be the main hindrance for its natural regeneration to maintain the wild

population. The genus, *Ceropegia* is under threat owing to either destructive collection or habitat degradation. Fifty species are present in India [20].Out of which 28 species are endemic to Peninsular India [21,22].

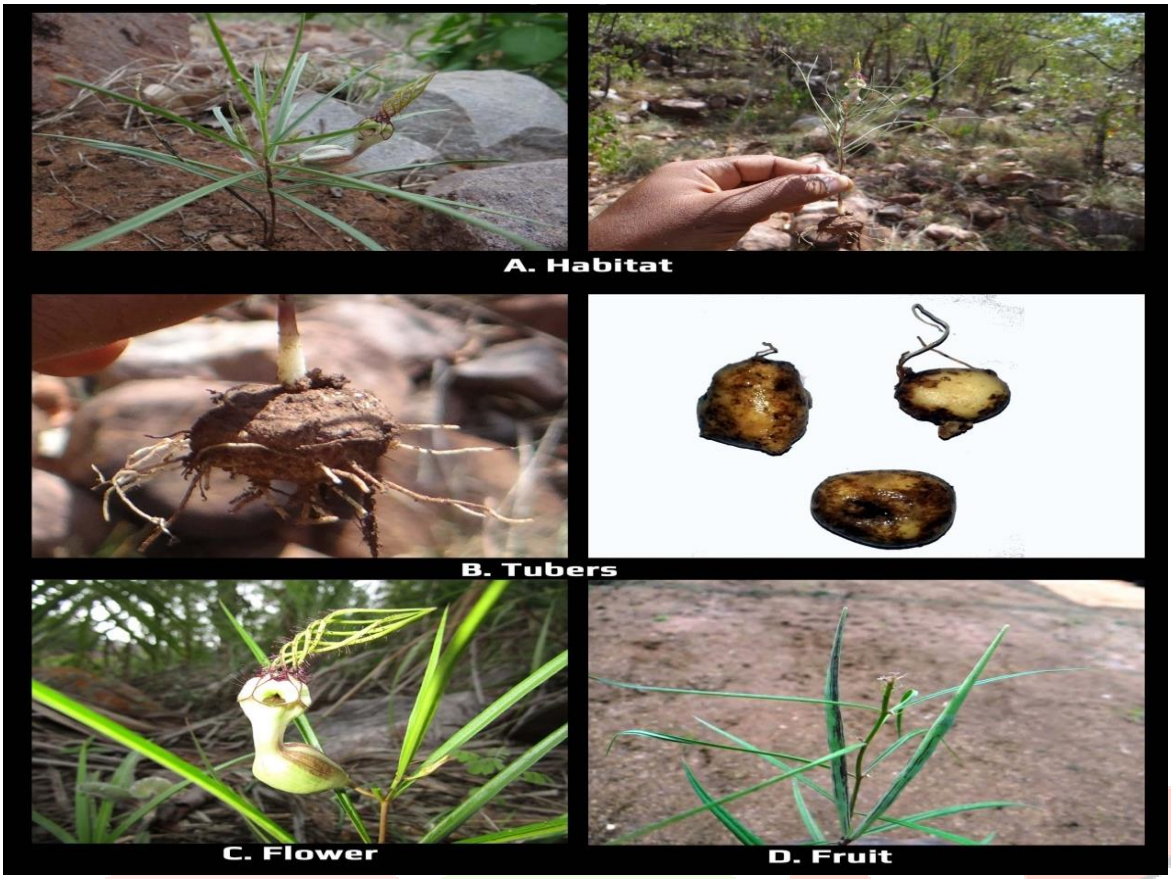



**Fig 1: *Ceropegia spiralis***

## MATERIAL AND METHODS

### Collection and Identification of Plant material:

*Ceropegia spiralis* tubers was collected from Jaapali area of Tirumala, Chittoor District, Andhra Pradesh, India; Herbarium specimen was identified and deposited (Voucher No.KP:13 ) in the Department of Botany, Sri Venkateswara University, Tirupati [23].

### Synthesis of CuONPs from *C. spiralis*:

Tubers were washed thrice with running tap water followed by Milli Pak pure water. The material was dried up to 10-15 days under shade conditions to evaporate residual moisture and finally ground with blender for further use. 5 g of finely grounded plant powder was extracted with 100 ml of Milli Q water boiled for 30 min and filtered with Whatmann no. 1 filter paper. An aliquot of 10 ml of aqueous plant extract was titrated with 100 ml of 5 mM CuSO4.5H2O for reduction of CuONPs at $50^0$C for 2 hrs. Obtained mixture was centrifuged at 10,000 RPM for 15 min to separate agglomerated, broad sized particles as well as plant admixtures [24].

**Characterization of CuONPs of *C. spiralis*:**

The green synthesized *C. spiralis* CuONPs were characterized by using different spectroscopic and microscopic tools. Initial confirmation of nanoparticles was done by UV–VIS spectroscopy (Nano drop 8000 UV-Vis -spectrometer) to know which metals of the phytochemicals were actually involved in the reduction of nanoparticles by surface Plasmon resonance method. Stabilization of nanoparticles; Fourier-Transform Infra Red (FT-IR) spectra of synthesized SNPs were analyzed in the range of 4,000 to 500 cm-1 with an IR-AFFINITY-1, IR by ATR method. Zeta potential of synthesized nanoparticles were analyzed to know the average size and stability of particles (Nanoparticle analyzer, Horiba SZ 100, Japan). XRD (Shimadzu, XRD-6000) was used to analyze crystalline nature and calculate the average size of particles. Microscopic analysis with TEM (HF-3300, 300 kV TEM/ STEM, Hitachi) instrument reveals the size, shape, dispersed nature and agglomerated pattern of nanoparticles [25-31].

**Antioxidant Activity [DPPH]:** DPPH (2,2-diphenyl-1-picryl hydrazyl) free radical scavenging method involves the stock solution prepared by dissolving 4 mg of DPPH in 100 ml of methanol and stored at 20 °C. 2 ml of this solution was added to 1 ml of *C. spiralis* tuber aqueous extract and *C. spiralis* CuONPs at different concentrations (25- 100µg/ml). Ascorbic acid was used as a standard. Where RSA is Radical scavenging activity, *Ac* is the absorbance of the control, and *As* is the absorbance of the sample or standard [32]

$$\text{Radical Scavenging Activity} = \frac{(\text{Ac}-\text{As})}{(\text{Ac})} \text{X100} \rightarrow (1)$$

**Antimicrobial studies of CuONPs**

Biosynthesized Copper oxide nanoparticles were analyzed for antimicrobial activity against three Gram-positive bacterial strains like *Staphylococcus aureus* MTCC-3160, *Enterococcus faecaulis* MTCC-2729, *Streptococcus pyogenes* ATCC 19615 and two Gram-negative bacterial strains like *Enterobacter aerogenes* MTCC-2822, and *Salmonella typhimurium* MTCC-3231. Antifungal studies were carried out in three fungal strains like, *Aspergillus niger* MTCC 281, *Candida albicans* MTCC-183 *C. tropicalis* MTCC-184, procured from Department of Botany, Bharathidasan University, Tiruchirappalli, Tamil Nadu. Disc diffusion assay method was carried out using standard protocol [33] Different concentrations (10,20,30,40, µg/ml) of Plant extract, SynthesizedNPs and Kanamycin/Flucanazole was applied on separate filter paper discs (Whatman No. 1) filter paper with 6 mm diameter), and allowed to dry before being placed on the agar medium. The *C. spiralis* tuber extract was used as positive controls, CuSO4 is negative control respectively. Kanamycin and Fluconazole (5mcg/disc) were used as standard controls for bacterial and fungal strains, respectively.

**Anticancer activity:**

CuONPs of *Ceropegia spiralis* was subjected to MTT 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide for colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow colored water soluble tetrazolium dye (MTT) to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan

crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportional to the number of viable cells and can be measured spectro photometrically at 570nm [34-35]. HeLa (Human Cervix Adenocarcinoma) cell line is procured from National Centre for Cell Sciences (NCCS), Pune, India. The Dulbecco's Modified Eagle's Medium with high glucose is used to growing up $2 \times 10^4$ cells per well in 96-well plates and incubated in 5% $CO_2$ atmosphere at $37^0$C for 24 h supplemented with 2 mM/L glutamine, 10% Foetal Bovine Serum (FBS) with 10 µg/ml of Ciprofloxacin [36].

Afterwards medium was expelled and treated with different concentrations (12.5, 25, 50, 100 and 200µl/ml) CuONPs of *C. spiralis* incubated for 24hrs. Further, remove spent media and add 100 of MTT reagent with the 0.5mg/ml concentration and incubate the plate for 2.5hrs for the reaction. Later, remove MTT reagent completely and add 100 µl of 100% Dimethyl sulfoxide (DMSO) to solubilize the formazone crystals completely and measure the absorbance at 570nm using 96 well Plate reader. (The 0.1% of DMSO used to dissolve the nanoparticles and set as negative control and 15 µM Camptothecin treated cell lines were set as positive control). The initial experiment was maintained for 0 to 24 h of timeline period with 12 h of time gap period to check probability of cell toxicity. It provides specific time course period to allow functional cell mortality to understand the experiment in a flexible and adaptable way. According to the results, significant cytotoxicity was observed at 24-hrs at $37^0$c incubation period. The percentage of cell viability was calculated by the following formula [37].

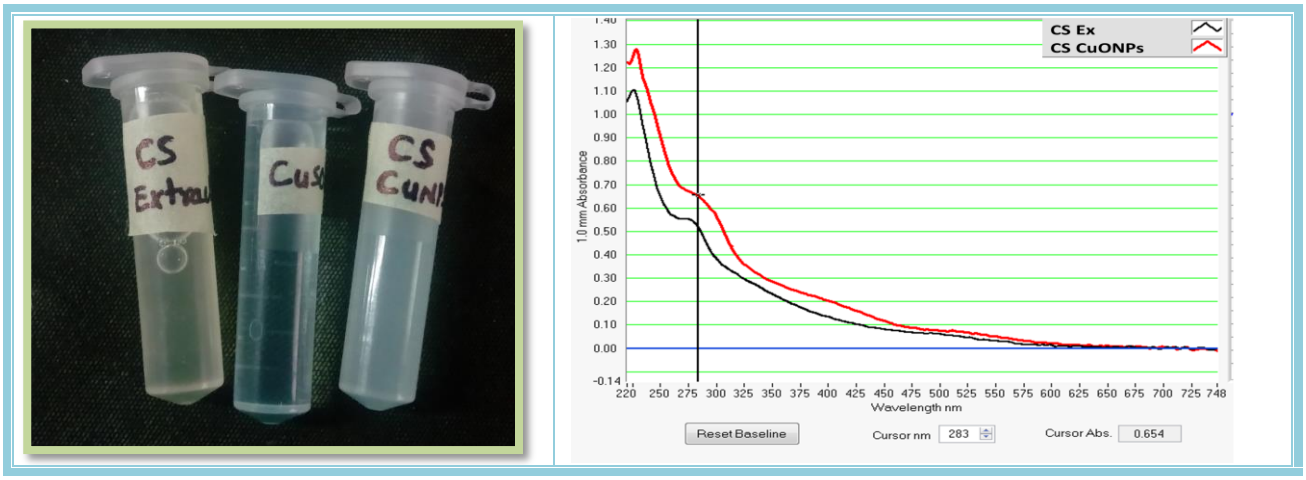
$$\text{Percentage of Cell viability} = \frac{\text{OD value of treated cell lines}}{\text{OD value of control}} \text{ X } 100 \quad \rightarrow (2)$$

**RESULTS:**

**Ultra Violet - Visible Spectroscopy of *Ceropegia spiralis* tuber extract - CuONPs:**

It is observed that tuber extract of ***C. spiralis*** mediated CuONPs manifest a color change from **gray** to **light blue** (**Figure 2**). The color change mechanism of the reaction mixture is due to a reaction between copper (II) sulfate pentahydrate and sodium hydroxide to form copper (II) hydroxide which reacts with the plant extract to give copper oxide. The time taking for the reaction mixture 2 hrs.

After the reduction of CuO material, they formed as spherical shaped CuO nanoparticles by the action of plant phytochemicals. Plant phytochemicals act as capping and stabilizing agents to give particular shape and to avoid agglomeration between the particles. When analyzed this sample with UV–Vis spectrophotometer between the scan range of 220–750 nm. Nanoparticles in the reaction mixture were excited by absorbing light at different wavelengths due to surface plasmon resonance (SPR) nature to give respective broad peak, represents the particular metal nanoparticles were actually reduced. Synthesized reaction mixture showed that a broad peak at **283 nm** corresponds to copper oxide, which confirms the synthesized nanoparticles were CuONPs.




**(a)** **(b)**

**Figure 2: (a)** Synthesized CuONPs of ***Ceropegia spiralis*** mixture Colour change gray to light blue **(b)** UV- VIS analysis of synthesized CuONPs shows peak at **283 nm.**

**Fourier Transform Infrared (FTIR) spectra analysis of *Ceropegia spiralis* CuONPs**

FT-IR study of biologically synthesized CuONPs of ***Ceropegia spiralis*** nanoparticles was analyzed within the scan range of 4000–500 $cm^{-1}$ to know the feasible phytochemicals responsible for capping and stabilization. The FTIR spectral analysis of CuONPs (**Figure 3**) showed intensive peaks at **3145.90 $cm^{-1}$** assigned for C-H (Stretch) bond of aromatics. **1635.64 $cm^{-1}$** assigned for N-H (Bend) bond of primary amines. **1207.44 $cm^{-1}$** assigned for C-N (Stretch) bond of aliphatic amines. **1072.42 $cm^{-1}$** assigned for C-O (Stretch) bond of alcohol. **866.04 $cm^{-1}$** assigned for C-Cl (Stretch) bond of alkyl halides. **607.58 $cm^{-1}$** assigned for –C=C-H (bend) bond of alkynes.

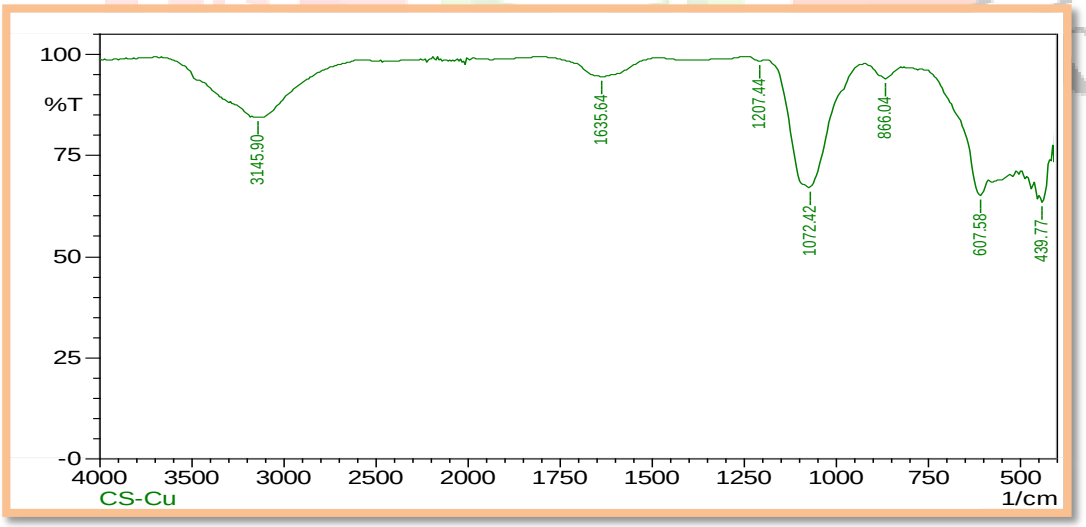



**Figure 3:** FTIR analysis of green synthesized CuONPs of ***Ceropegia spiralis.***

**Particle size and Zeta potential analysis of *C. spiralis* - CuONPs:**

The particle size of the biosynthesized CuONPs is detected by the intensity and laser diffraction method using the biosynthesized colloidal solution in which the ***C. spiralis*** CuONPs are polydispersed in mixture solution. The biosynthesized CuONPs was found to be **59.2 nm** (**Figure 4(A)**) with and PI (Polydispersed index) value **0.466**. Further the zeta potential analysis of ***C. spiralis*** CuONPs was detected as **-16.0 mV**

(**Figure 4(B)**), due to its negative zeta potential the CuONPs did not form agglomeration in the medium, leading to long term stability, because of the electrostatic repulsive force between the CuONPs. Zeta potential is an essential parameter for the characterization of stability in aqueous nanosuspensions. A minimum of ± 30 mV Zeta potential value is required for the indication of stable nanosuspension.

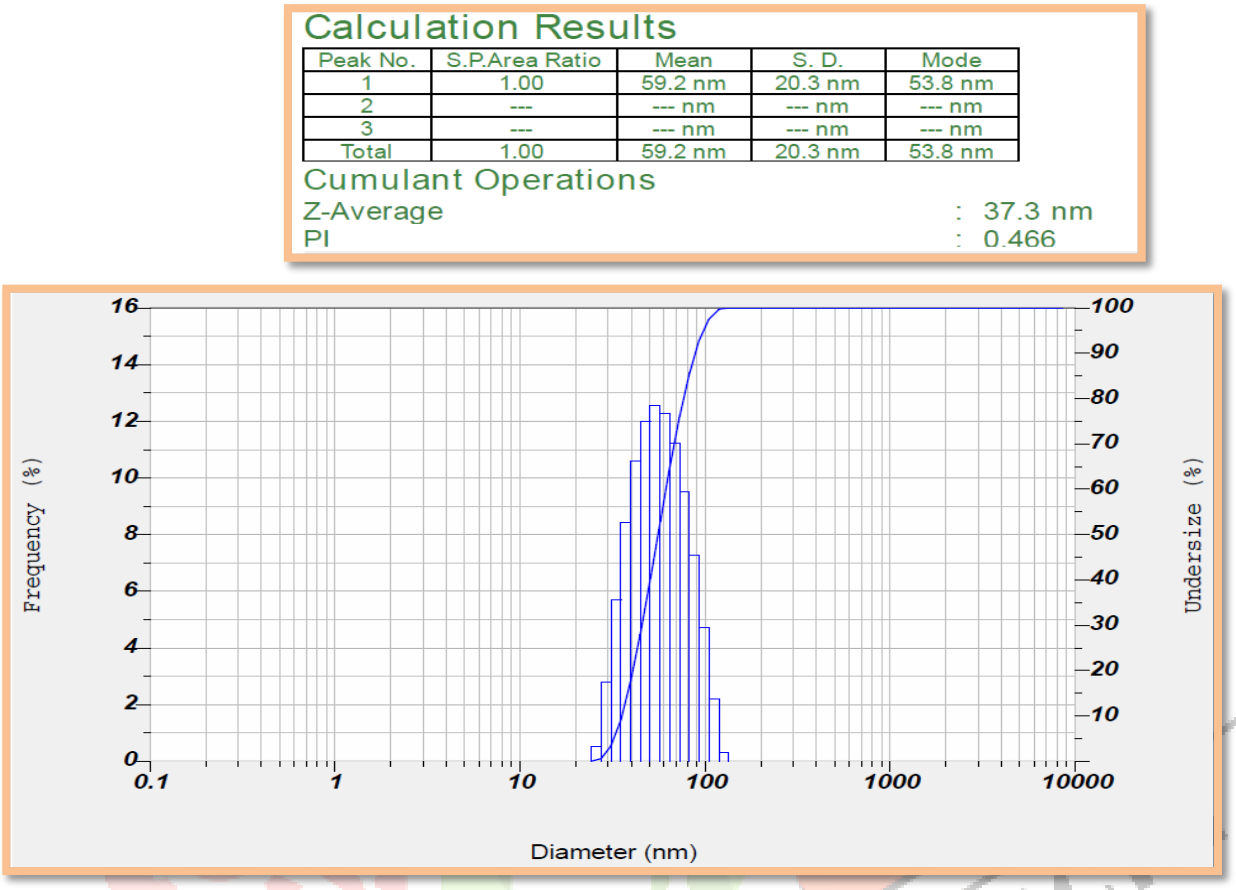



**Figure 4(A):** Particles size distribution curve for ***Ceropegia spiralis*** - CuONPs

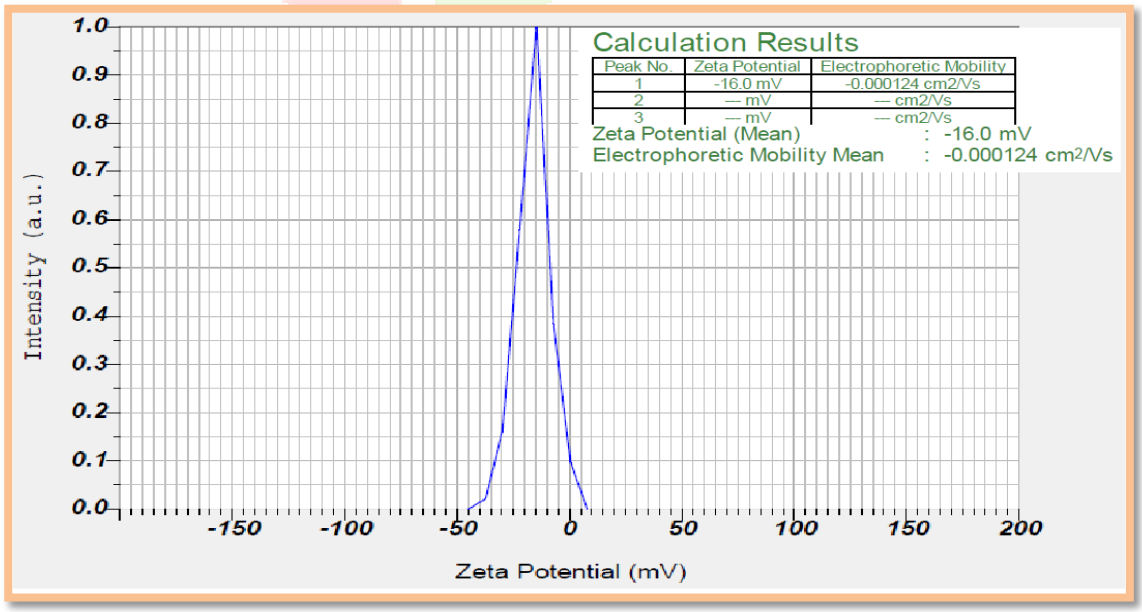



**Figure 4(B):** Zeta potential of green synthesized CuONPs of ***Ceropegia spiralis.***

**X-Ray diffraction analysis (XRD) of *Ceropegia spiralis* -CuONPs:**

X-Ray Diffraction analysis of synthesized CuONPs nanoparticles of ***Ceropegia spiralis*** showed 18 Bragg reflections at **15.69, 17.54, 19.00, 22.92, 24.20, 28.19, 29.51, 32.77, 33.78, 33.78, 37.32, 42.32, 43.60, 45.64, 47.62, 50.04, 51.64, 56.64 corresponds to 010, 101, 002, 111, 012, 112, 020, 103, 200, 103, 113, 014, 200, 220, 301, 024, 105, 230** integer integer 'hkl' planes, respectively. This indicates end-centered monoclinic crystalline nature of nanoparticles (**Figure 5**). This result was affirmed by cross checking the obtained data with JCPDS Card no. 781588, 710251.

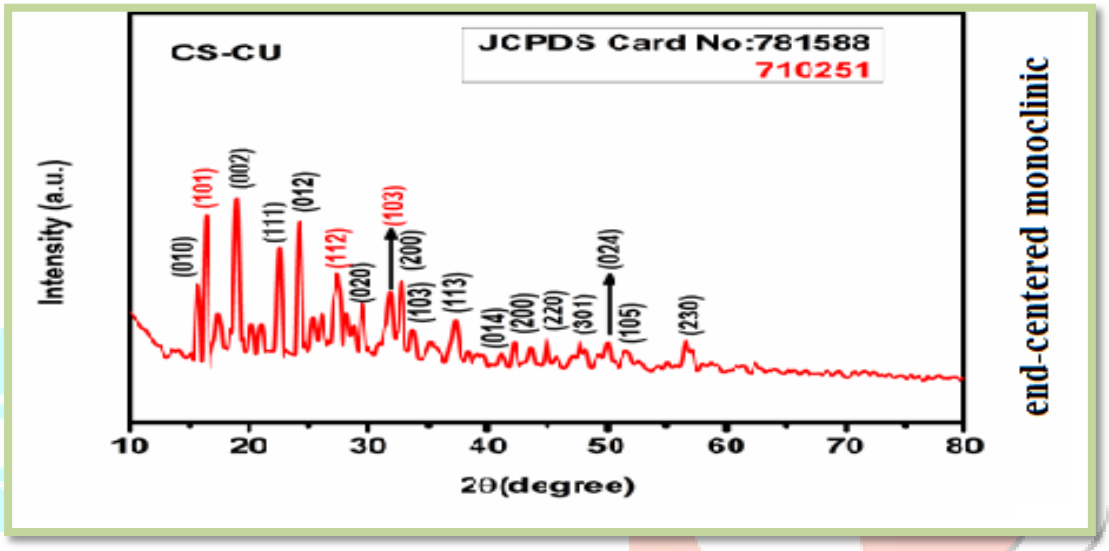



**Figure 5:** XRD pattern of green synthesized CuONPs of ***C. spiralis.***

**Transmission electron microscopy (TEM) analysis of *Ceropegia spiralis* – CuONPs**

The analyses of the TEM images are useful tools for measuring the size and shape of the synthesized nanoparticles. Morphological structure and distribution of synthesized copper nanoparticles characterized at high magnifications are coated on copper grids and analyzed by Hitachi HF- 3300 advanced with 300 kV.

Selected Area Electron Diffraction (SAED) pattern shows that the CuONPs nanoparticles of ***C. spiralis*** are **crystalline** in nature (**Figure 6(A)**). The **20 nm** resolution studies with TEM analysis revealed that the particles were **spherical** in shape, non-agglomerated, polydispersed. At the 100 nm resolution revealed that the particles with the size range from **1.8** to **3.48 nm**. This result indicates the synthesized nanoparticles were crystalline in nature.

**Particle analysis:**

Micrographs from TEM analysis showed **3.73 nm** average sizes of the particles with 0.99 adjacent 'R' square values calculated by using the following Gauss fitting formula (**Figure 6(B)**)

The average size of the nanoparticle was calculated as 3.73 nm. The size measured by DLS is slightly greater than HR-TEM because the DLS measured the hydrodynamic diameter. The result analysis confirmed that both HR-TEM and XRD analysis showed a similar average size of nanoparticles. The differences in the average size of nanoparticles are due to the preparation of sample time and variable instrumental conditions. However apart differences, the size measured by DLS and HR-TEM analysis showed similar results (**Table 1**).

**Table 1: Average size measured by DLS and HR-TEM.**

| DLS analysis | HR-TEM |
|---|---|
| 79.0 nm | 3.73 nm |

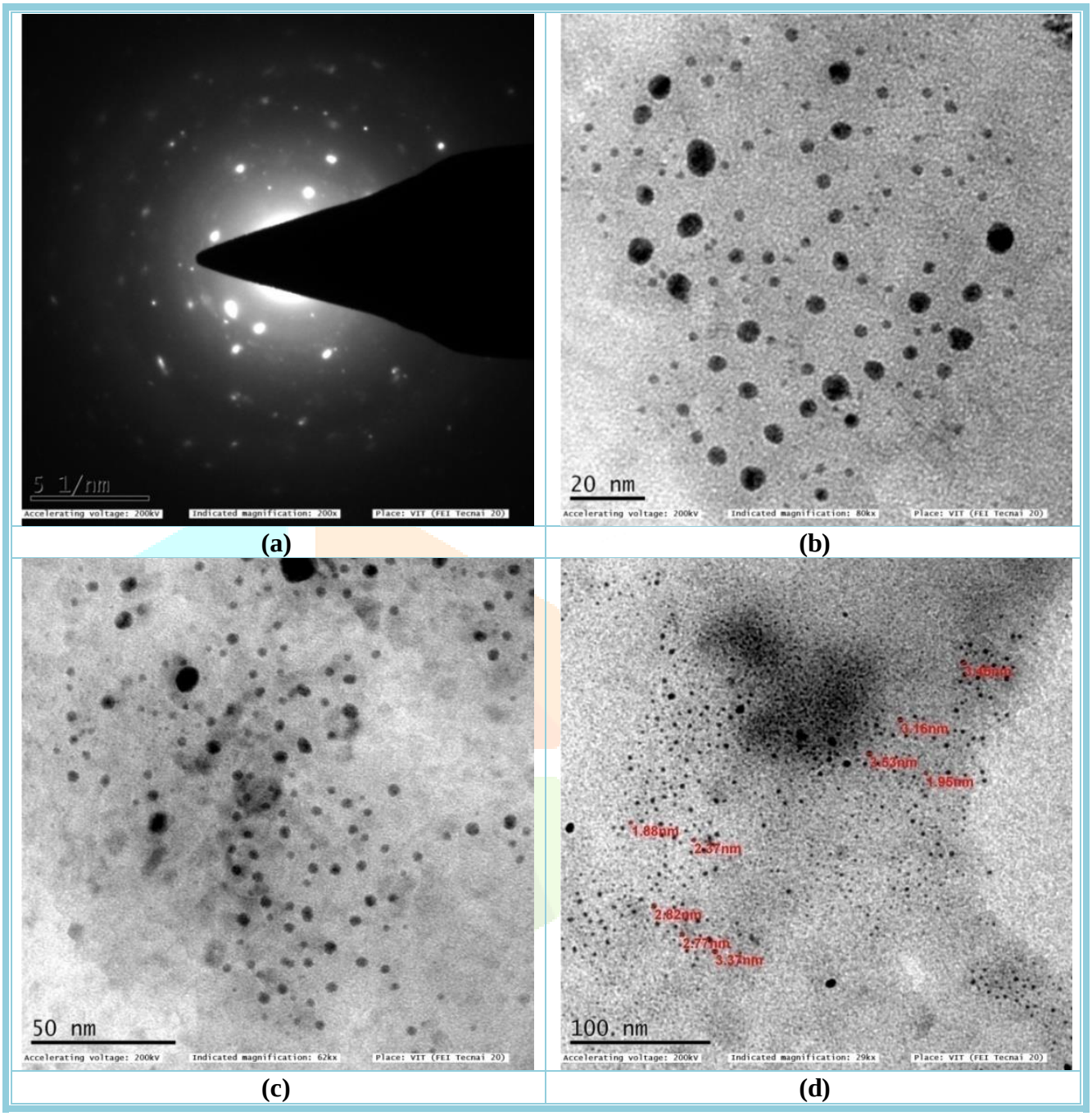



**Figure 6 (A):** TEM micrographs of biosynthesized of ***C. spiralis*** - CuONPs **(a)** Selected area electron diffraction (SAED) of green synthesized SNPs, **(b)** 20 nm resolution SNPs shows mostly spherical shaped nanoparticles. **(C)** 50 nm resolution shows mostly spherical shaped. **(D)** 100 nm resolution nanoparticles with **1.8 to 3.48** nm size.

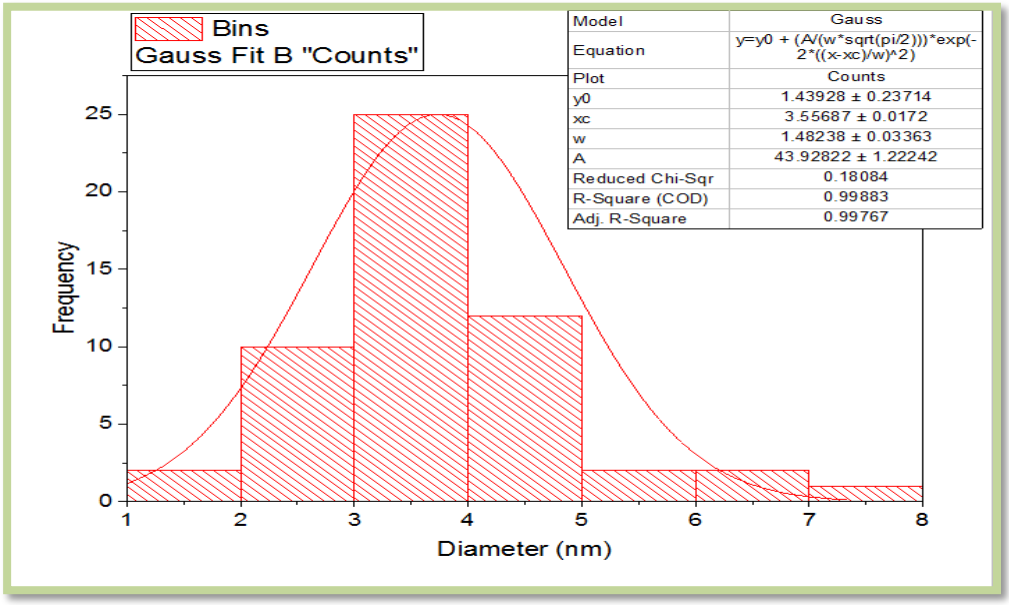



**Figure 6(B):** Particle size analysis of ***C. spiralis*** – CuONPs with Gaussian fitting formula represents average size of particles

**Energy Dispersive X-Ray Spectroscopy (EDX) of *Ceropegia spiralis* – CuONPs analysis:**

The EDAX analysis of synthesized of ***C. spiralis*** sample shows **51.65 %** weight percentage of **copper** metal along with **0.00% carbon, 25.31 % sodium, 17.3 % oxygen, 0.61 % sulfur, 0.57 % chlorine, 3.31 % potassium, 0.62 % magnesium** and **0.59 % calcium** indicates the sample having high purity of copper nanoparticles (**Table 2 and Figure 7**).

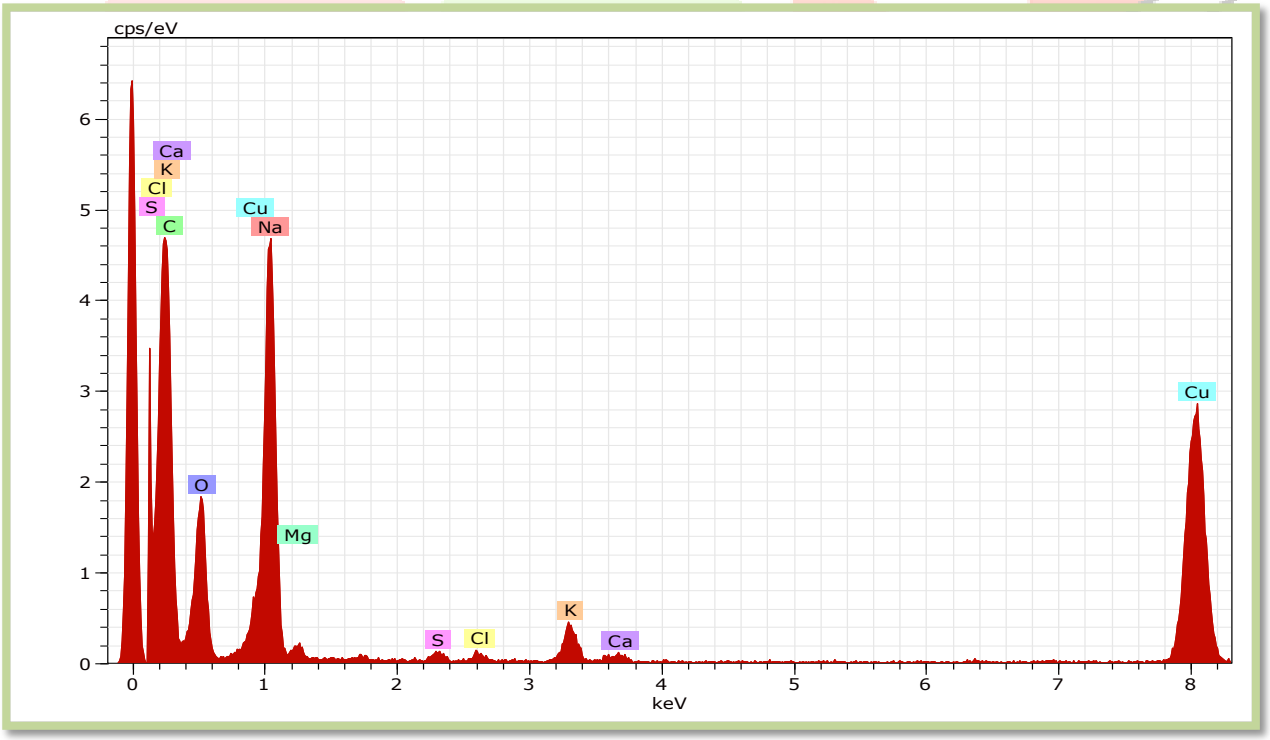



**Figure 7:** EDAX analyses of green Synthesized CuONPs of ***C. spiralis.***

**Table 2 :** EDX analysis of green synthesized CuONPs of ***C. spiralis***

| Elements | Series | Net | unn. C [wt. %] | corm. C [wt. %] | Atom. C [wt. %] | Error (3 sigma) [wt. %] |
|---|---|---|---|---|---|---|
| **Sodium** | K – series | 16722 | 25.31 | 25.31 | 34.88 | 2.43 |
| **Carbon** | K – series | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Oxygen** | K – series | 6278 | 17.33 | 17.33 | 34.31 | 1.77 |
| **Copper** | K – series | 20965 | 51.65 | 51.65 | 25.74 | 4.86 |
| **Sulfur** | K – series | 399 | 0.61 | 0.61 | 0.60 | 0.18 |
| **Chlorine** | K – series | 366 | 0.57 | 0.57 | 0.51 | 0.18 |
| **Potassium** | K – series | 2123 | 3.31 | 3.31 | 2.68 | 0.45 |
| **Magnesium** | K – series | 407 | 0.62 | 0.62 | 0.81 | 0.18 |
| **Calcium** | K – series | 357 | 0.59 | 0.59 | 0.47 | 0.19 |
| | | Total | 100.00 | 100.00 | 100.00 | |

**Antioxidant Analysis of *C. spiralis* - CuONPs:**

The synthesized CuONPs of ***C. spiralis*** showed better antioxidant potential when compare to standard ascorbic acid by DPPH scavenging assay method. The antioxidant activity was increased in dose-dependent manner. The highest percentage activity was exhibited at 100 µg/ml concentration of ***C. spiralis*** CuONPs (66.01%) ***C. spiralis*** and with aqueous extract (56.32%) < Ascorbic acid (74.44%) (**Table 3** and **Figure 7 (A and B)**) From the results, it is concluded that Copper oxide nanoparticles of ***C. spiralis*** possess good DPPH activity when compared to that of ***C. spiralis*** aqueous extract. The antioxidant activity of CuONPs by the DPPH method shows a strong absorption band at 517 nm.

**Table 3:** Antioxidant activity of biosynthesized ***C. spiralis*** – CuONPs

| Concentration | Aqueous Extracts (%) | CuONPs (%) | Ascorbic Acid (%) |
|---|---|---|---|
| 25 µg/ml | 14.02 ± 0.08 | 25.18 ± 0.3 | 47.45 ± 0.16 |
| 50 µg/ml | 24.24 ± 0.3 | 33.08 ± 0.36 | 56.26 ± 0.42 |
| 75 µg/ml | 36.14 ± 0.18 | 57.64 ± 0.71 | 66.25 ± 0.45 |
| 100 µg/ml | 56.32 ± 0.2 | 66.01 ± 0.54 | 74.44 ± 0.42 |

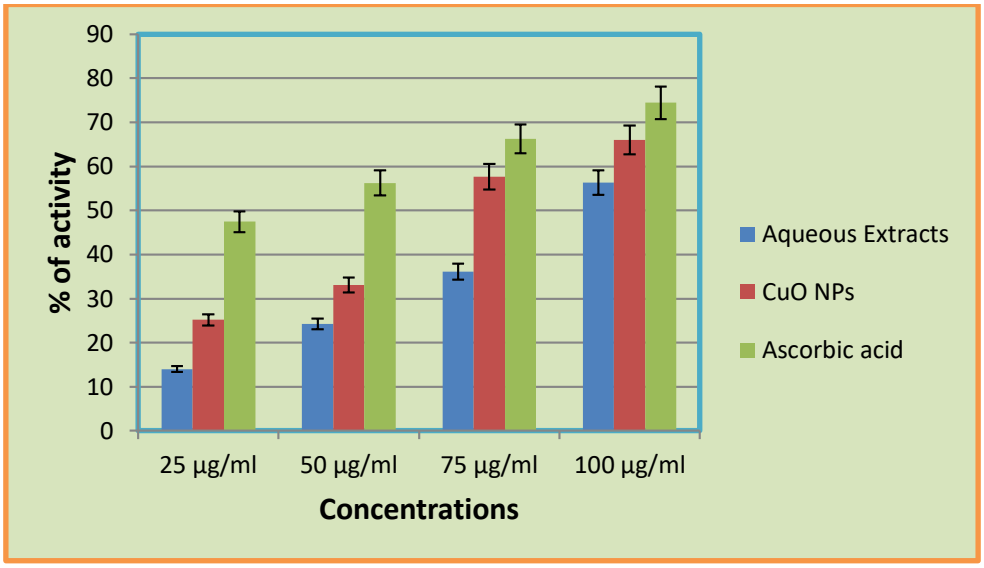



**Figure 7(A):** Antioxidant activity of biosynthesized ***C. spiralis*** – CuONPs

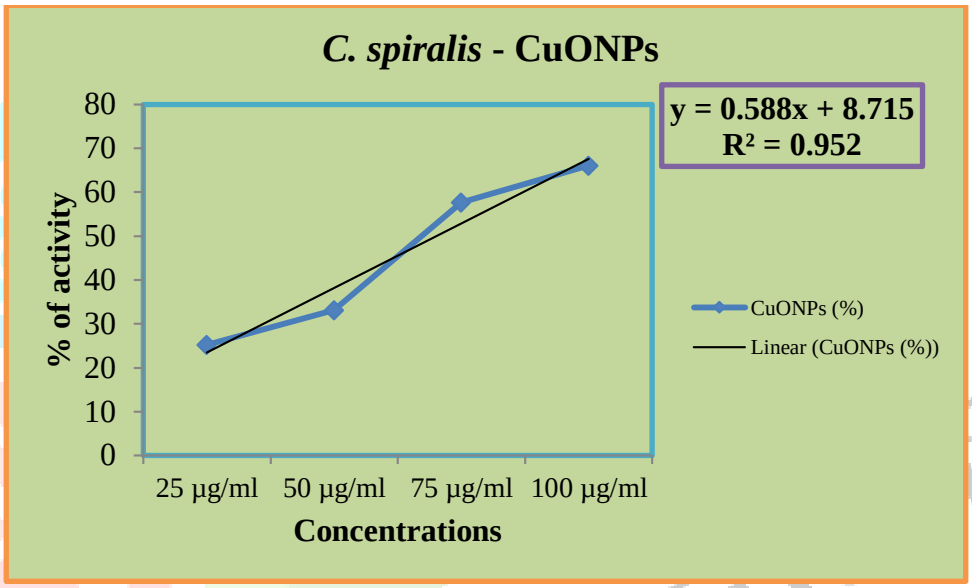



**Figure 7 (B):** Antioxidant activity of biosynthesized ***C. spiralis*** – CuONPs

## Antimicrobial activity of *C. spiralis* – CuONPs:

Antimicrobial studies of green synthesized of ***C. spiralis*** CuONPs has shown growth inhibitory results on different microorganisms. The highest zone of inhibition was observed on ***Enterobacter aerogenes*** with **17 mm** followed by ***Staphylococcus aureus*** with **13.33 mm** gram positive bacterial strains (**Table 4 and Figure 8(A and B)**). Whereas on fungi the highest zone of inhibition was observed on ***Candida tropicalis*** with **14.25 mm** (**Table 5 and Figure 9 (A and B)**). In this study, the zone of inhibition was observed less in fungi when compared with bacteria.

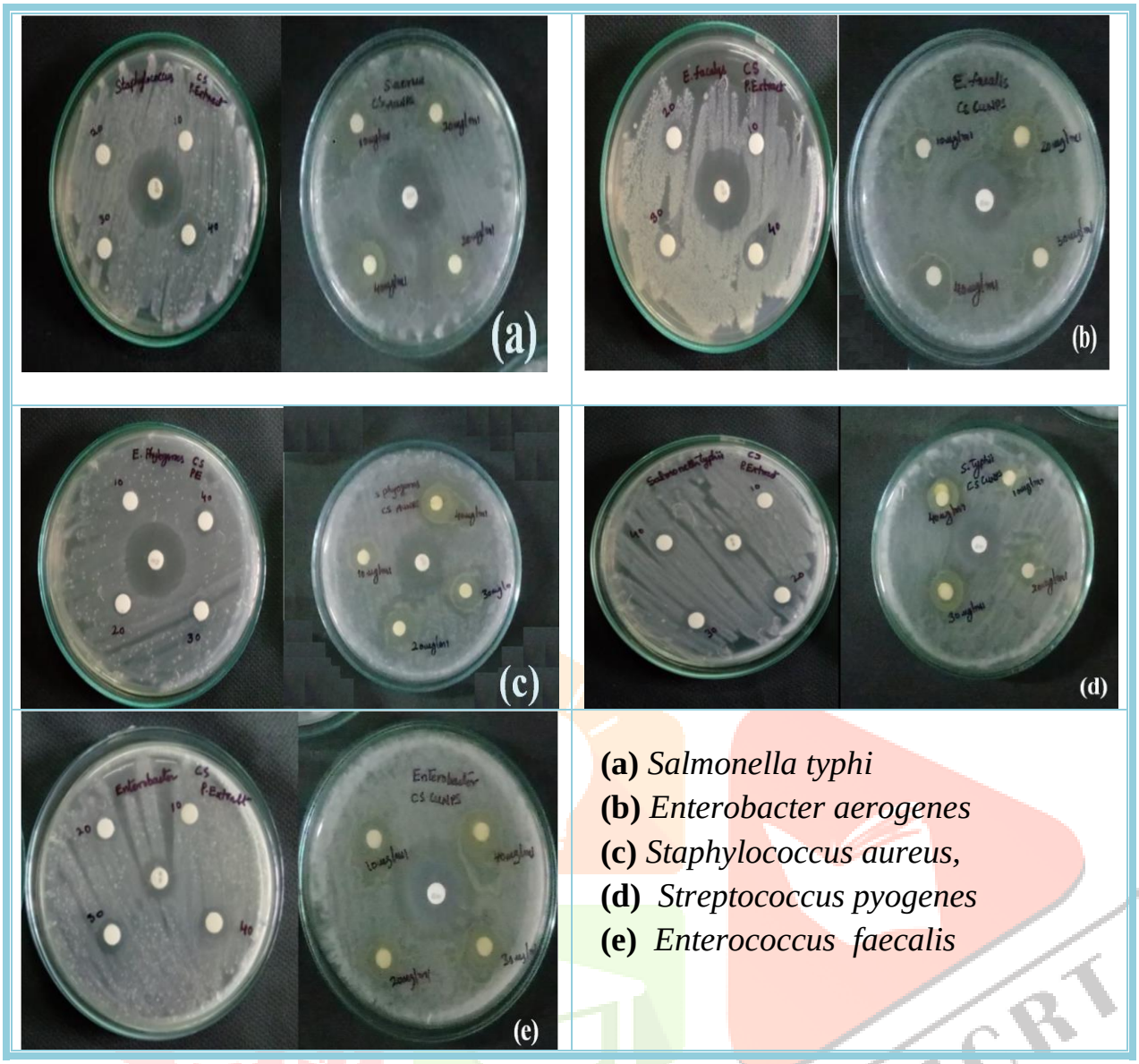



**Figure 8(A):** Antibacterial activity of biosynthesized of ***C. spiralis*** - CuONPs against Grem +ve the and Grem –ve bacteria

**Table 4:** Effect of different extracts and green synthesized ***C. spiralis*** – CuONPs on clinically isolated bacterial Strains**.**

| Concentration (µg/ml) | *Streptococcus pyogenes* | | *Enterococcus faecalis* | | *Staphylococcus aureus* | | *Salmonella typhi* | | *Enterobacter aerogenes* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Zone of Inhibition (mm) | | | | | | | | | |
| | PE | CuONPs | PE | CuONPs | PE | CuONPs | PE | CuONPs | PE | CuONPs |
| **10 (µg/ml)** | 6.67 ± 0.33 *** | 10.67 ± 0.33 *** | 7.33 ± 0.33 *** | 7.67 ± 0.33 *** | 7.33 ± 0.33 *** | 6.67 ± 0.33 *** | 7.67 ± 0.33 *** | 8.33 ± 0.33 *** | 7 ± 0 *** | 10 ± 0.58 *** |
| **20 (µg/ml)** | 11.33 ± 0.67 *** | 10.00 ± 0.58 *** | 11.67 ± 0.33 *** | 10.33 ± 0.33 *** | 8.33 ± 0.33 *** | 10.67 ± 0.33 *** | 12.67 ± 0.33 | 11.33 ± 0.33 *** | 10 ± 0.58 ** | 11.33 ± 0.33 *** |
| **30 (µg/ml)** | 11.67 ± 0.67 *** | 13.67 ± 0.33 *** | 12.33 ± 0.33 *** | 11.00 ± 0.58 *** | 9.67 ± 0.33 *** | 12.33 ± 0.33 *** | 13.67 ± 0.33 | 12.00 ± 0.58 *** | 12.33 ± 0.33 | 12 ± 0.58 *** |
| **40 (µg/ml)** | 13.33 ± 0.33 *** | 11.67 ± 0.33 *** | 14.67 ± 0.67 *** | 13.67 ± 0.33 *** | 12.33 ± 0.33 *** | 13.33 ± 0.67 *** | 14.33 ± 0.33 ** | 13.00 ± 0.58 *** | 13.33 ± 0.33 | 17.00 ± 0.58 ** |
| **Kanamycin** | 23.33 ± 0.67 | 19.00 ± 0.58 | 22.67 ± 0.33 | 18.00 ± 0.58 | 23.67 ± 0.33 | 18.00 ± 0.58 | 12.33 ± 0.33 | 18.33 ± 0.33 | 12.67 ± 0.33 | 20.67 ± 0.88 |

All the data are expressed as **mean ± SEM:** *** p<0.01, ** p<0.02, * p<0.03 as compared to Control group, n=4; (One – way ANOVA followed by Dunnett's test).

**Antibacterial activity of *C. spiralis* – CuONPs:**

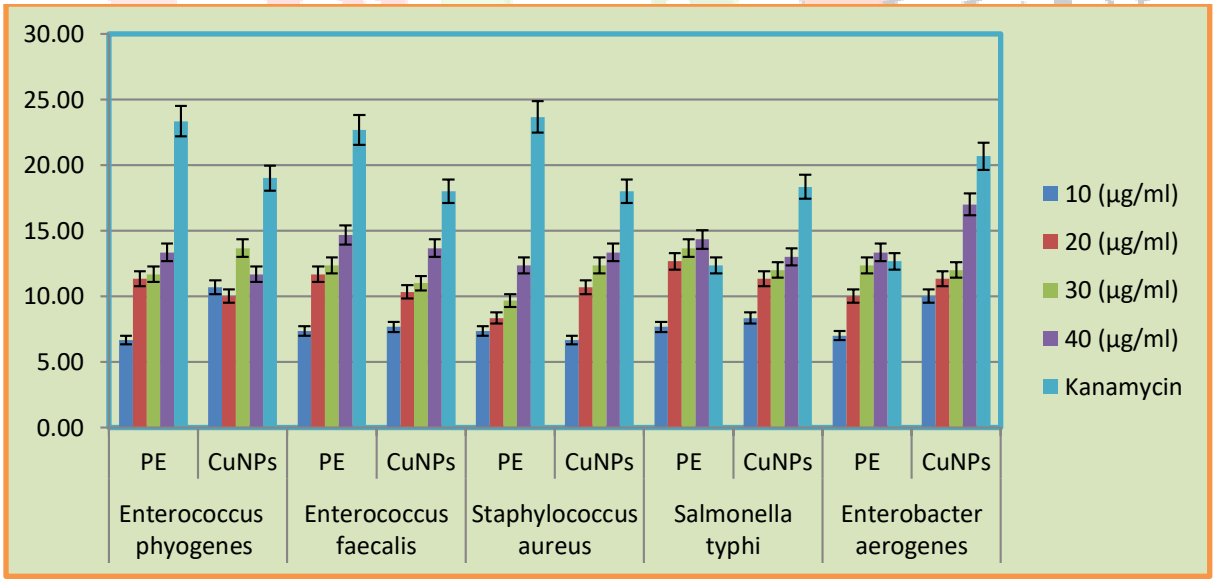



**Figure 8(B)**: Zone of inhibition of ***C. spiralis*** – CuONPs and different extracts bacterial strains

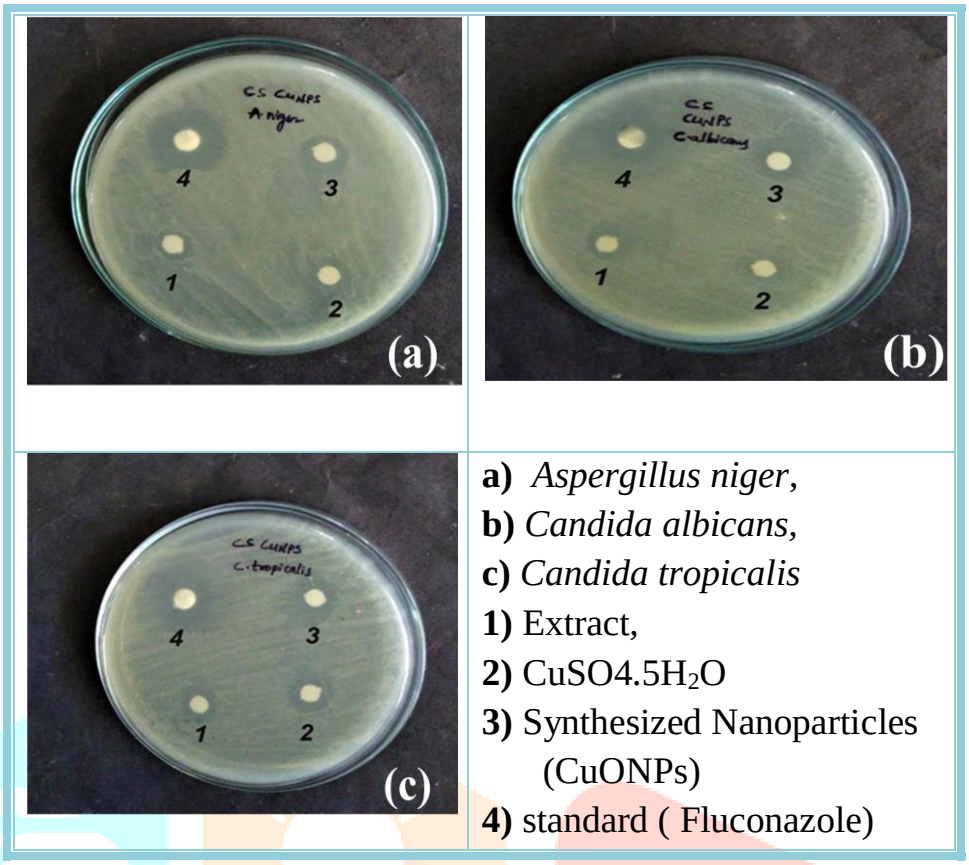



**Figure 9(A):** Antifungal activity of ***C. spiralis*** – CuONPs

**Table 5:** Effect of different extracts and green synthesized ***C. spiralis*** – CuONPs on Fungal strains.

| Name of the Organism | Extracts | CUSO4.5H2O | CuONPs | Fluconazole |
|---|---|---|---|---|
| **Aspergillus niger** | 8.75 ± 0.25 *** | 11.5 ± 0.29 *** | 13.5 ± 0.29 *** | 18.75 ± 0.25 |
| **Candida albicans** | 8 ± 0 *** | 10 ± 0 *** | 12 ± 0.41 *** | 15.75 ± 0.25 |
| **Candida tropicalis** | 9.5 ± 0.29 *** | 12.5 ± 0.5 *** | 14.25 ± 0.25 *** | 20.5 ± 0.29 |

All the data are expressed as **mean ± SEM:** *** $p<0.01$, ** $p<0.02$, * $p<0.03$ as compared to Control group, n=3; (One – way ANOVA followed by Dunnett's test).

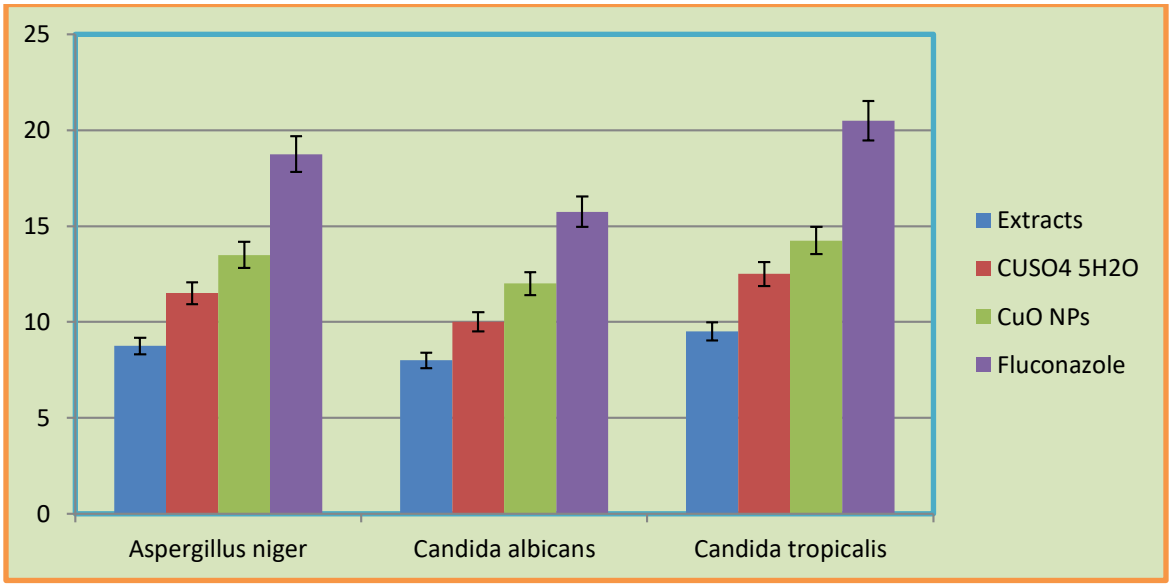
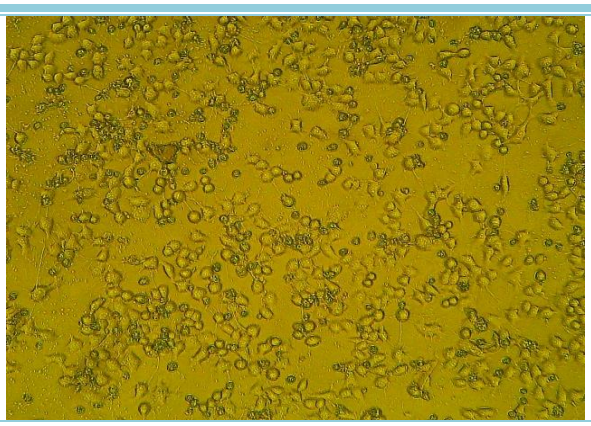



**Figure 9 (B):** Zone of inhibition of ***C. spiralis*** – CuONPs and different extracts on fungal strains.

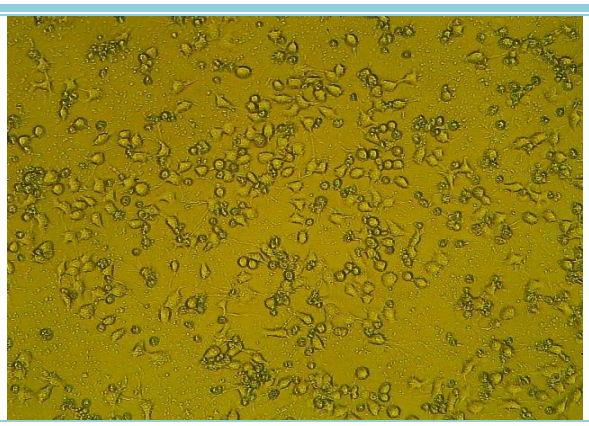
**Anticancer activity *C. spiralis* – CuONPs:**

The HeLa cell lines (Human Cervix Adencarcinoma) were used for cytotoxicity analysis by reading formazan crystals formed by the reaction of mitochondrial dehydrogenase (MTT) assay. At 48 h of time course incubation period, a significant abatement in cell viability was observed in the treated cell lines, while the concentration of CuONPs was increased from 12.5, 25, 50, 100 and 200 µg/ml. Dimethyl sulfoxide (DMSO) was used as a positive control to exhibit 100% of healthy proliferated cells (**Table 6 and Figure 10 (A, B )**). The 50 µg/ml concentrations ($IC_{50}$) of CuONPs have the capability to reduce 50% of treated cell lines when compared with negative control. The cytotoxicity of nanoparticles may depend on the small size and spherical shape of the particles [38]

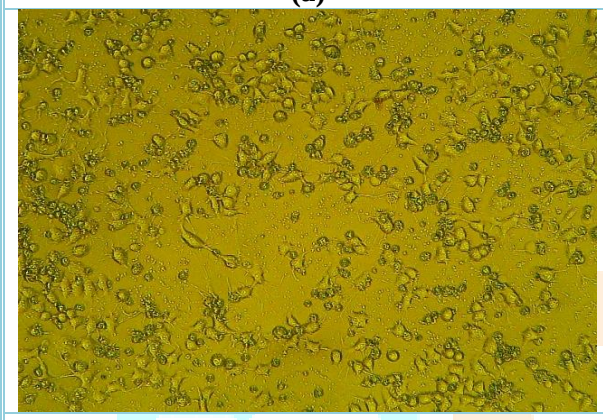
Anticancer activity of CuONPs exhibited potential towards HeLa cancer cell lines, shows **75.17%** cell death with cell viability **24.83%**. (IC50 value 72.42 µg/ml). But, there is no report on CuONPs synthesized from any medicinal plant to attribute anticancer activity against HeLa cell lines. From this study, the green synthesized CuONPs from tuber extract showed 3.73 nm size, spherical shaped particles, which exhibit strong cytotoxic activity against HeLa cell lines [39].



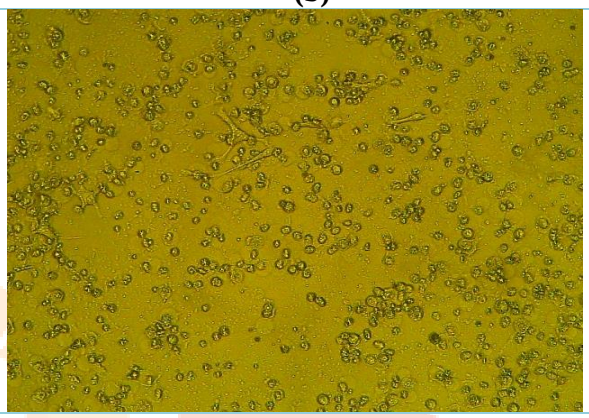
(a)



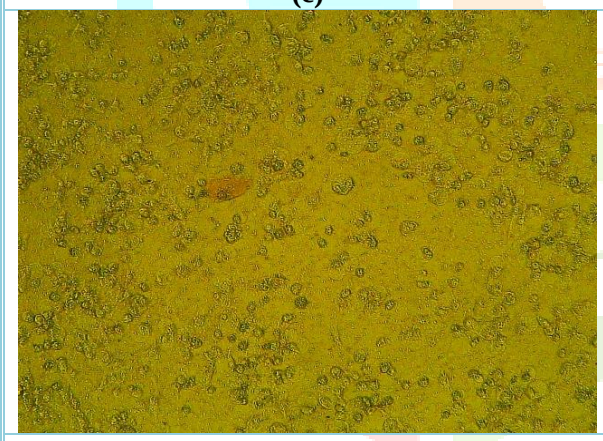
(b)



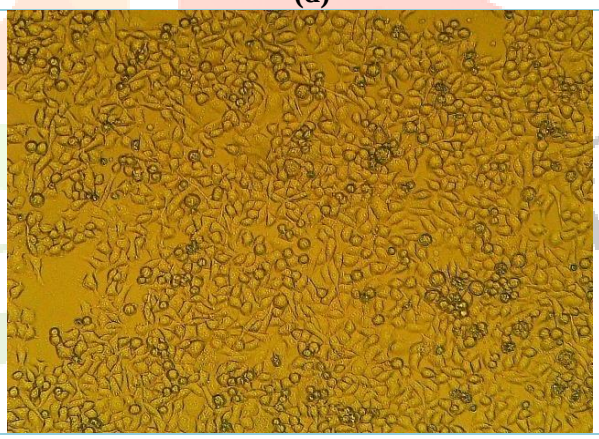
(c)



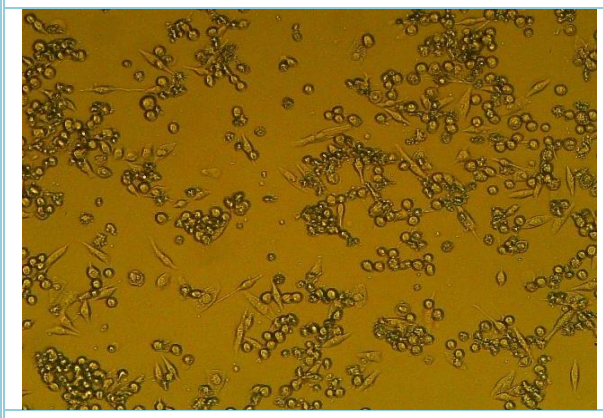
(d)



(e)



(f)



(g)

**(a)** 12.5µg/ml,
**(b)** 25 µg/ml,
**(c)** 50 µg/ml,
**(d)** 100 µg/ml,
**(e)** 200 µg/ml,
**(f)** DMSO (0.1%) negative control,
**(g)** Camptothecin (5.2 µg/ml) positive control.

**Figure 10(A):** Anticancer activity of synthesized ***C. spiralis*** - CuONPs.

**Table 5:** Anticancer effect of ***Ceropegia spiral*** CuONPs on He La cell line (Human Cervix Aden carcinoma) by MTT assay.

| S. No | Concentrations (µg/ml) | Absorbance (O.D) | Cell viability (%) | Cell Death (%) |
|---|---|---|---|---|
| **1** | **DMSO** | 0.86 | 100 | 100 |
| **2** | **12.5** | 0.7645 | 88.89 | 11.11 |
| **3** | **25** | 0.65 | 75.58 | 24.42 |
| **4** | **50** | 0.505 | 58.72 | 41.28 |
| **5** | **100** | 0.3785 | 44.1 | 55.9 |
| **6** | **200** | 0.2135 | 24.83 | 75.17 |
| **7** | **Camptothecin** | 0.4425 | 18.54 | 81.46 |

**Anticancer activity from *C. Spiralis* – CuONPs**

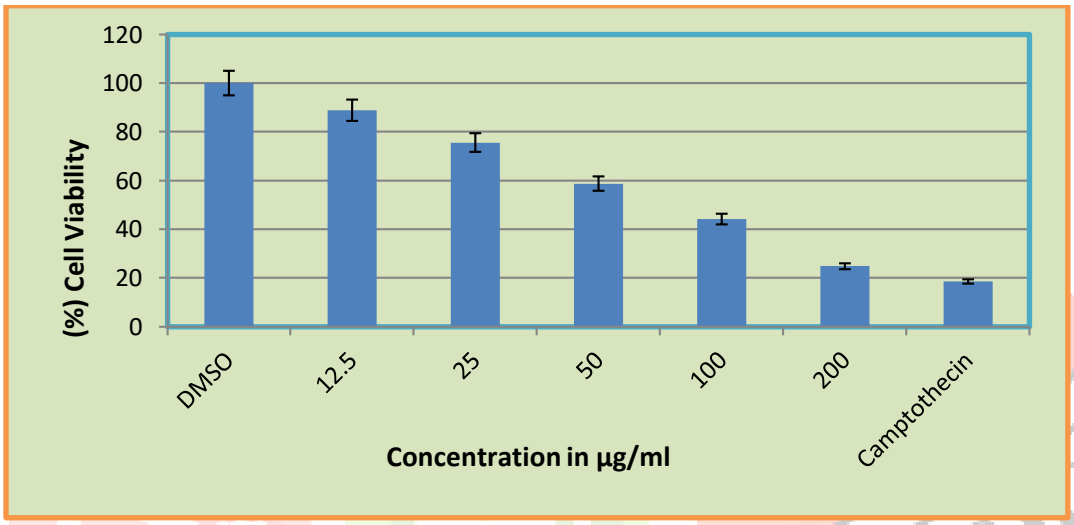



**Figure 10 (B):** Anticancer activity of ***Ceropegia spiralis*** CuONPs.

## DISCUSSION

Copper oxide nanoparticles antifungal activity was observed less when compared to antibacterial activity may be due to fungal cell walls are made up of chitin, a fibrous substance comprising of polysaccharides having N-acetyl glucosamine and a nitrogen group, which is more firm to allow the passage of nanoparticles from the outer layer of the cell wall to the inner layer. In the case of bacteria, cell membranes are made up of peptidoglycon a polymer having sugars and amino acids, which is less firm and passage of nanoparticles is easy when compared with fungi.

Size, shape and agglomeration pattern of the nanoparticles depend on quantity based presence of phytochemicals in the medicinal plants. This might be useful in reducing, capping and stabilization of nanoparticles to narrow size with spherical shape [40]. Pharmacological activities of *Ceropegia* species has been subjected to various investigations. The therapeutic importance of the genus *Ceropegia* is mainly due to the presence of '*cerpegin'*, a pyridine alkaloid. DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity, ferric reducing antioxidant power (FRAP) as well as metal chelating ability of major phenolic

compounds such as gallic acid, vanillin, cathechol and ferulic acids from the leaves of *Ceropegia* species such as *C. spiralis, C. panchganiensis* and *C. evansii* has been reported [41]. The leaves of *C. bulbosa* showed the highest superoxide dismutase activity [42]. *C. juncea* has possessed various potent secondary metabolites such as tannins, flavonoids and many polyphenolic compounds. Ethanolic leaf extracts of *C. juncea* showed gastroprotective and antioxidant activities in rats due to the presence of polyphenolic compounds [43]. Antibacterial activity of three *Ceropegia* species such as *C. spiralis, C. juncea* and *C. candelabrum* var. *candelabrum* on human pathogens *Klebsiella pnemoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas* that ethanol extract showed a higher antibacterial activity as compared to chloroform and aqueous extracts [44]. Antimicrobial activity with whole plant extract of *Ceropegia pusilla* against five bacterial strains *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, *Shigella sonnei, Bacillus* sp.and four fungal strains *Candida albicans, Aspergillus* sp., *Penicillium* sp., *Mucor* sp. [45]. Comparative studies on *in vivo* and *in vitro* tuber extracts of *C. pusilla* confirmed Antiproliferative property against HeLa cancer cell line [46]. The three *Ceropegia* species C. *spiralis, C. juncea* and *C. candelabrum*, screened for anti-cancer activity and confirmed the potent anticancer effect of ethyl acetate fraction of *C. spiralis* against cell lines namely HCT-118 (Colon cancer cell) [47].

**CONCLUSION:**

Hence the present study, reported as cost effective, eco-friendly, green approach method for production of CuONPs from the tuber extract of *C. spiralis.* The colour change pattern and surface Plasmon resonance spectra of UV–VIS data 283 nm confirms the formation of SynthesizedNPs. Phenols and Proteins are mainly responsible for reduction and stabilization of these SNPs revealed by FTIR. Zeta potential analysis revealed that – 16.0 mV of negative value indicates greater stability of particles. XRD analysis revealed that end-centered monoclinic crystalline nature of nanoparticles. Microscopic analysis with TEM showed spherical shaped particles with a size range from **1.8** to **3.48 nm**. These particles were mostly settled in non-agglomerated and poly-dispersed condition.

The expository synergistic efficiency of CuONPs showed growth inhibitory activity against microorganisms as well as HeLa (Human Cervix Adenocarcinoma) Cancer cell lines. Further studies need to be performed to evaluate the molecular mechanism behind the anticancer potential of the CuONPs against the Human Cervix cancer cells. The presence of higher concentrations of phenols and proteins in the tuber may be the reason behind the formation of narrow sized particles, bestowed to antimicrobial and anticancer activity. This is the first report on phyto-synthesized CuONPs from the tuber extract of *C. spiralis.* This study may pave a way for the studies of CuONPs further pharmacognostic studies against anticancer, antibacterial and antifungal drug designing.

**ACKNOWLEDGEMENT:**

The authors are highly thankful to UGC-BSR for providing meritorious research fellowship and highly grateful to DST-PURSE, Sri Venkateswara University- Tirupati, VIT-Vellore for providing technical support.

**REFERENCES**

**[1]. R. Emmanuel, M. Saravanan, M. Ovais, S. Padmavathy, Z. K. Shinwari, and P. Prakash (2017):** Antimicrobial efficacy of drug blended biosynthesized colloidal gold nanoparticles from *Justicia glauca* against oral pathogens: A nano antibiotic approach Microb. Pathog. **113, 295–302**.

**[2]. S. Pirtarighat, M. Ghannadnia, and S. Baghshahi (2019):** Green synthesis of silver nanoparticles using the plant extract of *Salvia spinosa* grown in vitro and their antibacterial activity assessment *J. Nano struct. Chem.* 9, 1–9.

**[3]. H. Agarwal, S. V. Kumar, and S. Rajeshkumar (2017):** A review on green synthesis zinc oxide nanoparticles an eco-friendly approaches *Resour. Effic. Technol*. 3, 406–413.

**[4]. S. A. Aromal, V. K. Vidhu, and D. Philip (2012):** Green synthesis of well dispersed gold nanoparticle using macrotyloma uniflorum Spectrochim. Acta *A Mol. Biomol. Spectrosc*. 85, (1), 99–104.

**[5]. H. Barabadi, M. Ovais, Z. K. Shinwari, and M. Saravanan (2017):** Green synthesis of silver nanoparticles using *Alysicarpus monilifer* leaf extract and its antibacterial activity against MRSA and CoNS isolates in HIV patients Green Chem. Lett. Rev. 10, (4), 285–314.

**[6]. P. Prakash, P. Gnanaprakasam, R. Emmanuel, S. Arokiyaraj, and M. Saravanan (2013):** Green synthesis of silver nanoparticles from leaf extract of *Mimusops elengi*, Linn. for enhanced antibacterial activity against multidrug resistant clinical isolates *Colloids Surf. B Biointerfaces* 108, 255–259.

**[7]. A. K. Mittal, Y. Chisti, and U. C. Banerjee (2013):** Synthesis of metallic nanoparticles using plant extracts *Biotechnol*. Adv. 31, 346–356.

**[8]. M. Anandan, G. Poorani, P. Boomi, K. Varunkumar, K. Anand, A. A. Chuturgoon, M. Saravanan, and H. G. Prabu (2019):** Plant-Mediated Synthesis, Characterization and Bactericidal Potentialof Emerging Silver Nanoparticles Using Stem Extract of *Phyllanthu spinnatus: A Recent Advance in Phyto nanotechnology Process Biochem* 1481-88.

**[9]. D. Horwat, D. Zakharov, J. L. Endrino, F. Soldera, A. Anders, S. Migot, R. Karoum, P. H. Vernoux, and J. F. Pierson (2011):** Microstructure of sputter-deposited noble metal-incorporated oxide thin films patterned by means of laser inter ference *J. Coat. Technol*. 205, S171–S177.

**[10]. H. R. Naika, K. Lingaraju, K. Manjunath, D. Kumar, G. Nagaraju, D. Suresh, and H. Nagabhushana (2015):** Green synthesis of CuO nanoparticles using *Gloriosa superba* L. extract and their antibacterial activity J. Taibah Univ. Sci. 9, 7–12.

**[11] Rajgovind,Gaurav Sharma1, Deepak Gupta Kr, Nakuleshwar Dut Jasuja and Suresh Joshi C (2015):** *Pterocarpus marsupium* Derived Phyto-Synthesis of Copper Oxide Nanoparticles and their Antimicrobial Activities. *J Microb Biochem Technol* Volume 7(3): 140-144.

**[12] P.C. Nagajyothi, P. Muthuraman , T.V.M. Sreekanth , Doo Hwan Kim, Jaesool Shim (2017):** Green synthesis: In-vitro anticancer activity of copper oxide nanoparticles against human cervical carcinoma cells *Arabian Journal of Chemistry* 10, 215–22.

**[13] Rohit Guin, Shakila Banu A, Gino A Kurian (2015):** Synthesis Of Copper Oxide Nanoparticles Using *Desmodium Gangeticum aqueous* Root Extract *International Journal of Pharmacy and Pharmaceutical Sciences* Vol 7, Suppl 1, 60-65.

**[14] Elavarasan Nagaraj Kokila Karuppannan Prakash Shanmugam Sujatha Venugopal (2019):** Exploration of Bio-synthesized Copper Oxide Nanoparticles Using *Pterolobium hexapetalum* Leaf Extract by Photo catalytic Activity and Biological *Evaluations Journal of Cluster Science* 30:1157–1168.

**[15] Rangacharyulu (1991):** Floristic studies of Chittoor district, Ph.D thesis, S V. University, Tirupati.

**[16] Nayar MP, Sastry ARK, (1987):** Red data book of Indian plants. Calcutta: Botanical Survey of India.

**[17] Jain SK, Defilips RA (1991):** Asclepiadaceae. In: Algonae MI, editor.Medicinal plants of india.USA: Reference Publication Inc, 144-152.

**[18] Kirtikar KR and Basu BD (1935):** Indian medicinal plants 3.New Delhi: Bishen singh mahendrapal singh.

**[19] Adibatti NA ,Tirugnanasambantham P, Kulothugan C, Viswanatha S, Kameshwaran L, BalakrishnaK, Sukumar E A (1991):** pyridine alkaloid from *Ceropegia juncea. Phytochemistry* 30 (7):2449-2450.

**[20] Surveswaran S. Kamble M.Y. Yadav S.R. and Sun M (2009):** Molecular phylogeny of *Ceropegia* (Asclepiadoideae, Apocynaceae) from Indian Western Ghats. *Plant Syst. Evol*. **281**: 51-63.

**[21] Ahmedulla M. and Nayar M.P (1986):** Endemic plants of the Indian region peninsular India. Bot. Survey India: The Kolkata.

**[22] Ansari M.Y (1984);** Asclepiadaceae: Genus *Ceropegia* - Fascicles of Flora of India. Botanical Survey of India, Calcutta, **16**: 1-34

**[23] Jain S K and, Rao R R (1997):** A Handbook of Field and Herbarium Today and Tomorrow Printers and Publishers, New Delhi.

**[24] Sadia Saif, Arifa Tahir, Tayyaba Asim and Yongsheng Chen (2016):** Plant Mediated Green Synthesis of CuO Nanoparticles: Comparison of Toxicity of Engineered and Plant Mediated CuO Nanoparticles towards *Daphnia magna*, *Nanomaterials* 2016, 6, 205

**[25] Mulvaney P (1996):** Surface plasmon spectroscopy of nanosized metal particles. Langmuir; 12:788–800.

**[26] Chandran S P., Chaudhary R., Pasricha A., Ahmad A.,and Sastry M. (2006);** "Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract," *Biotechnology Progress,22*(2), 577–583.

**[27] Skoog DA., James Holler F., Timothy A.,Nieman (1998):** Principles of instrumental analysis

**[28] Sathyanarayana (1996);** Small-pore aluminium phosphate molecular sieves with chabazite structure. Incorporation of magnesium in structures -34 and -44, *Journal of Chemical Society.*

**[29] Aruldhas (2001); Characterisation of stoichiometric sol–gel mullite by fourier transform infrared spectroscopy,** *International Journal of Inorganic Materials Volume 3, Issue 7*, Pages 693-698

**[30] Cullity B D**, **Stock S R(2001);** *Elements of X-ray Diffraction, 3rd Ed., Prentice-Hall Inc.*

**[31] Klug H.P, Alexander L.E (1974):** *X-ray diffraction procedures* ; Wiley: New York.

**[32] Sharma O P and Bhat T K (2009):** DPPH antioxidant assay revisited, Food Chemistry; 113(4): 1202-05.

**[33] Cruickshank R (1986):** Medical microbiology: a guide to diagnosis and control of infection. *Livingston publishers, Edinburghand London*.

**[34] Alley M C, Scudiere D A, Monks A., Czerwinski, M, Shoemaker R II, and Boyd M R (1986):** Validation of an automated microculture tetrazolium assay (MTA) to assess growth and drug sensitivity of human tumor cell lines. *Proc. Am. Assoc. Cancer Res.*, 27: 389.

**[35] Mosmann, T. J. Immunol (1988)**. *Methods Cancer Res*. 48: 589-601, 1988. 65: 55-63, 1983.

**[36] Jagadeesh M, Rashmi H K, Subba Rao Y, Sreenath Reddy A, Prathima B, Uma Maheswari Devi P (2013):** Synthesis and spectroscopic characterization of 3,4-difluoroacetophenonethiosemicarbazone and its palladium (II) complex: evaluation of antimicrobial and antitumour activity. *Spectrochim Acta A Mol Biomol Spectrosc* 115:583–587

**[37] Kadirareddy R H, Vemuri S G, Palempalli U M (2016):** Probiotic conjugated linoleic acid mediated apoptosis in breast cancer cells by down regulation of *NFjB. Asian Pac J Cancer Prev* 17:3395–3403.

**[38] Park MV, Neigh AM, Vermeulen JP, de la Fonteyne LJ, Verharen HW, Briede JJ et al (2011)** The effect of particle size on thecytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. *Biomaterials 32:9810–9817.*

**[39] Sivaraj R, Rahman PK, Rajiv P, Narendhran S and Venckatesh R (2014):** Biosynthesis and characterization of *Acalyphaindica* mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. *SpectrochimActa A MolBiomolSpectrosc. 14:255–258.*

**[40] Saif S, Tahir A, Asim T, Chen Y (2016):** Plant mediated green synthesis of CuO nanoparticles: comparison of toxicity of engineered and plant mediated CuO nanoparticles towards Daphnia magna. *Nanomaterials 6:1–15.*

**[41] Karayil S, Veeraiah K(2014).** Phytochemical analysis of *Ceropegia juncea* (Roxb.): Traditionally used Medicinal plant. Int J Innov Res Dev. 2014; 3(4):192-199.

**[42] Rama Murthy K, Kondamud R, Chandrasekhara Reddy M, Karuppusamy S, Pullaiah T (2012)**. Check-list and conservation strategies of the genus *Ceropegia* in India. *Int J Biodiv Conserv*. 2012; 4(8):304-315.

**[43] Venu P, Prasad K, Kaliamoorthy S and H Huber (2017)** (Apocyanaceae: Asclepiadoideae) on the verge of extinction. *Current Science.* (2017); 112:2189-2191.

**[44] Binish T, Ben CP, Paul Raj K (2014).** *In Vitro* plant regeneration and antibacterial activity studies on three endemic species of *Ceropegia. Int J Pharma Bio Sci.;* 5

**[45] Suresh D and Paulsamy S (2010)** Phenological observation and population dynamics of six uncommon medicinal plants in the grasslands of Nilgiris, Western Ghats, India. Int J Sci Technol. 2010; 4(02):185-192.

**[46] Kalimuthu K, Prabakaran R, Brindha C. (2014)** Angiogenesis and Antioxidant Activity of *in vitro* and *in vivo* Tuber of *Ceropegia pusilla* Wight and Arn. Br J Pharm Res. 2014; 4(5):608-616.

**[47] Binish T, Mary Suja R. 2015** Determination of *in vitro* anti proliferative effect of three important *Ceropegia* species ethanolic extracts on cultured hct-118 cell lines. Int J Pharm Bio Sci. 2015; 6(1):899-904.