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Synthesis and Characterization of Copper Oxide Nanoparticles from Aqueous Extract of *Cyanotis tuberosa* (Roxb.) Schult. & Schult. F. and Analysis of Antioxidant, Antimicrobial and Anticancer Activity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cyanotis tuberosa (Roxb.) Schult. & Schult.f family Commelinaceae is traditionally used as ethnomedicine for curing several health problems in India. In this study copper oxide CuONPs were synthesized using *C. tuberosa* tubers aqueous extract. The biosynthesized CuONPs were characterized using an Ultra violet–visible spectroscopy, Fourier-Transform Infra Red spectroscopy, X-RAY Diffraction Analysis, Scanning electron microscopy. The results obtained from SEM showed that the copper oxide nanoparticles were a face-centered cubic crystal structure. The characterization of the biosynthesized CuONPs through EDS also indicated that the reaction product was composed of highly pure CuO NPs. The invitro antimicrobial activity of different concentrations of green synthesized copper oxide nanoparticles of C*. tuberosa* showed that the highest inhibition zone was observed on *Klebsiella pneumonia* (15.25 mm). Furthermore, the antioxidant activity of green synthesized CuONPs of C*. tuberosa* tubers aqueous extract was also tested the highest percentage activity was exhibited at 100 µg/ml CuONPs (56.32). The human

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cancer cell line was used for cytotoxicity analysis at 48 hrs of incubation period, a significant abatement in cell viability was observed against the treated cell lines. The study concludes that the C*. tuberosa* tubers aqueous extract can be successfully used for the synthesis of CuONPs that exhibit effective in vitro anti-bacterial, antioxidant and anticancer activity.

Keywords: Cyanotis tuberose; green synthesis; CuONPs; anticancer; antioxidant; antimicrobial.

1. INTRODUCTION

Metal nanoparticles play a crucial role in various fields, most importantly in the fields of medicine and pharmacy. Since few decades metal nanoparticles made of Ag and Au have been used in the field of medicine [1,2,3]. The search for novel nanoparticles with potential biological properties is going on in many fields. Recent studies explored the importance of CuO nanoparticles and their potential in the fields such as gas sensors, catalyst, solar energy conversion and photovoltaic devices along with medical field [4,5,6]. Though CuO nanoparticles show excellent activity, their synthesis plays is a key role in their function. Several methods either physical or chemical are available for the synthesis of CuO nanoparticles such as sonochemical methods, thermal oxidation route, thermal decomposition, hydrothermal approach, precipitation methods and reverse micro emulsion methods [7,8,9,10,11,12]. All of these methods have their own drawbacks such as toxic chemicals usage, expensive to afford, hazardous materials and labor engagement. The toxicity produced through these methods contaminates the environment. Most reliable and ecofriendly method of synthesis of CuO nanoparticles is biological method where plant extracts can be employed to synthesize the nanoparticles. The nanoparticles synthesized through biological means have plenty of applications in medical and clinical fields. CuO nanoparticles have been synthesized using microorganisms such as bacteria, fungi, yeast and by using several types of leaf extracts such as neem leaf extract, tea leaf extract, coffee powder extract and aloe vera extracts [13,14,15]

Recent reports have shown the synthesis of CuO nanoparticles using plant extracts and their applications in medical field. Copper nanoparticles synthesized from the aqueous extracts of black bean showed in-vitro anti cancer activity that can stimulate apoptosis and suppress the HeLa cells proliferation. Enhanced antibacterial activity against the microbes responsible for Urinary Tract Infections (UTI) by the copper nanoparticles prepared from cellulose gum was reported [16]. Numerous studies have been reported the antibacterial function of copper nanoparticles against different bacteria such as *Enterococcus faecalis*, *Shigella flexneri*, *Salmonella typhimurium*, *Proteus vulgaris*, *Staphylococcus aureus*, *Klebsiella pneumonia* [17], and *Aeromonas hydrophila*, *Flavobacterium branchiophilum* and *Pseudomonas fluorescens*, the most prominent fish pathogens [14]. Along with the copper nanoparticles other metal nanoparticles such as iron oxide and zinc oxide have been used in medical field for their antibacterial and anticancer activity [18,19]. The present study was aimed to study the biological functions of copper nanoparticles synthesized by the aqueous extracts of *Cyanotis tuberosa*.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

C. tuberosa fresh tubers were collected from Talakona area of Chittoor District, Andhra Pradesh, India; Herbarium specimen was identified and deposited (Voucher No.GR:02) in the Department of Botany, Sri Venkateswara University, Tirupati for future reference [20].

2.2 Synthesis of CuONPs from *C. tuberosa*

The tubers collected were washed thrice with running tap water to remove the contaminants followed by Milli Q ultra – purified water. The material was dried up to 10 - 15 days under shade conditions. The dried material was finally ground with blender for further use. 5 g of finely ground plant powder was mixed with 100 ml of Milli Q ultra – purified water. boiled for 30 min and filtered with Whatmann no. 1 filter paper. The filtrate thus collected was stored in dark bottle in the refrigerator. This filtrate was now used as aqueous extract of *C. tuberosa*. An aliquot of 10 ml of aqueous plant extract was titrated with 100 ml of 5 mM $CuSO_4.5H_2O$ for reduction at 50^oC for 2 hrs. The obtained mixture was then centrifuged at 10,000 rpm for 15 min to separate the agglomerated, broad sized particles and plant admixtures [21].

A. Habitat **B. Tubers** **C. Flower**

Fig. 1. Morphology of *Cyanotis tuberosa*

2.3 Characterization of CuONPs of *C. tuberosa*

The CuONPs synthesized with aqueous extract of *C. tuberosa* were characterized by using different spectroscopic and microscopic methods. 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 was 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 [22,23,24,25,26,27,28].

2.4 In-vitro Antioxidant Activity

Free radical scavenging activity of aqueous extract of *C. tuberosa* was determined by its ability to bleach the stable 2, 2 – diphenyl – 1 - picryl hydrazyl (DPPH) radical. The stock solution of DPPH free radical was prepared by dissolving 4 mg of DPPH in 100 ml of methanol and stored at 20 °C. 2 ml of this stock solution was added to 1 ml of *C. tuberosa* aqueous extract and *C. tuberosa* CuONPs separately at different concentrations (25 – 100 µg/ml). Ascorbic acid was used as a standard. The radical scavenging activity was calculated by using the formula

$$\text{RSA} = \frac{(\text{Ac}-\text{As})}{(\text{Ac})} \text{X}100$$

Where RSA is Radical scavenging activity, *Ac* is the absorbance of the control, and *As* is the absorbance of the sample or standard [29].

2.5 Antimicrobial Studies

The antimicrobial activity of the CuONPs synthesized with aqueous extracts of *C. tuberosa* was analyzed against bacterial and fungal strains. The bacterial strains include two Gram positive bacterial strains namely *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-731) and two Gram negative bacterial strains namely *Escherichia coli* (MTCC-443), *Klebsiella pneumoniae* (MTCC-741). The fungal strains include *Aspergillus niger* (MTCC 281), and *Candida albicans* (MTCC-183). The antibacterial activity was determined by disc diffusion method [30]. The samples include rhizome extract as positive control, 5 mM copper sulphate as negative control, *Streptomycin*/*Flucanozole* (10 mg) as standard and CuONPs as test sample. The sterile discs of size 6 mm were prepared from Whatman No. 1 filter paper and these sterile discs were impregnated with 20 µl of each sample on separate discs with the help of micropipette and allowed to dry for one hour under aseptic conditions. Freshly prepared nutrient agar plates were spreaded with the microbial cultures and the discs impregnated with

samples were placed on the plates and incubated at 37 °C for 24 to 48 h. After the incubation the zone of inhibition was measured. The experiment was carried out in triplicates [31].

2.6 Anticancer Activity

CuONPs of *C. tuberosa* tubers aqueous extract 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 spectrophotometrically at 570 nm [32,33]. MDA MB 453 (human breast cancer cell line) 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 × 104 cells per well in 96-well plates and incubated in 5% CO2 atmosphere at 370C for 24 h supplemented with 2 mM/L glutamine, 10% Foetal Bovine Serum (FBS) with 10 μg/ml of *Ciprofloxacin* [34]. Afterwards medium was expelled and treated with different concentrations (12.5, 25, 50, 100 and 200 µl/ml) CuONPs of *C. tuberosa* incubated for 24 hrs. Further, remove the spent media and add 100 µl of MTT reagent with the 0.5 mg/ml concentration and incubate the plate for 2.5 hrs 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 570 nm 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 hrs of timeline period with 12 hrs 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 370 C incubation period. The percentage of cell viability was calculated by the following formula [35].

$$\text{Percentage of Cell viability} = \frac{\text{OD value of treated cell lines}}{\text{OD value of control}} \text{ X } 100$$

3. RESULTS AND DISCUSSION

3.1 UV Spectroscopy of CuONPs *of C. tuberosa*

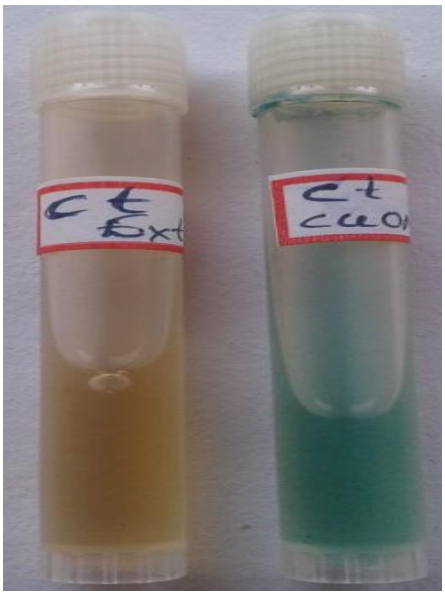
The brown colored fresh aqueous tuber extract of *C. tuberosa* has an ability to convert copper oxide solution into the ionic form. In this reaction, the tuber extract reacts with copper oxide solution and changes to green colour. The SPR spectrum peak of biosynthesized *C. tuberosa* CuONPs was obtained at 270 nm (Fig. 2).

3.2 FTIR Spectrum of *C. tuberosa* CuONPs

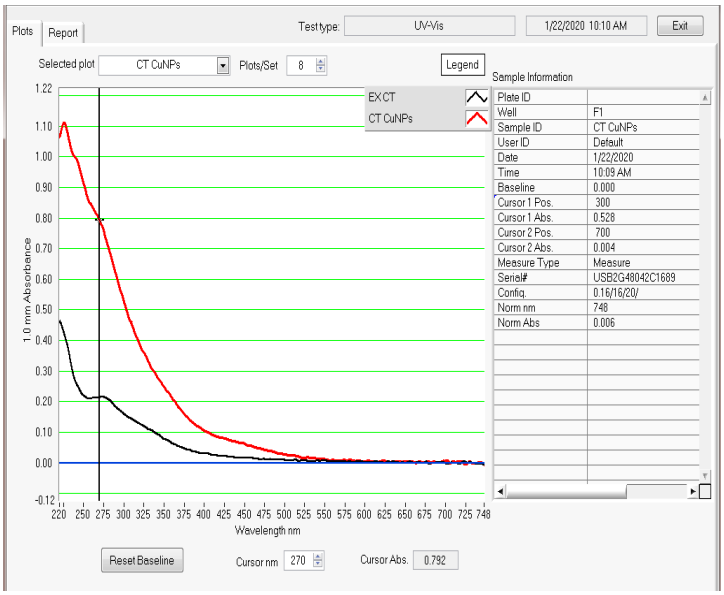
The FTIR analysis of tuber extract of *C. tuberosa* shows different peaks corresponding to different stretches of bonds present in the sample. The peaks include 3363.86 cm^{-1} assigned for – O – H (Stretch free hydroxyl) bond of phenols, 2681.75 cm^{-1} assigned for C – H (Stretch) bond of alkanes, 1639.49 cm^{-1} assigned for C – H (rock) bond of alkanes, 1109.07 cm^{-1} assigned for C – N (Bend) bond of alkenes, 1026.13 cm^{-1} assigned for C – N (Stretch) bond of aliphatic amines, 864.11 cm^{-1} assigned for C – H (oop) bond of aromatic, 777.31 cm^{-1} assigned for C – Cl (Stretch) bond of alkynes, 555.50 cm^{-1} assigned for C –Br (Stretch) bond for alkyl halides.

Table 1. FTIR Spectrum of *C. tuberosa* CuONPs

S. No.	Wave number	Molecular Motion	Vibration/ Intensity	Functional Group
1.	3363.86 cm^{-1}	– O – H	Stretch	Phenols
2.	2681.75 cm^{-1}	C – H	Stretch	Alkanes
3.	1639.49 cm^{-1}	C – H	Rock	Alkanes
4.	1109.07 cm^{-1}	C – N	Bend	Alkenes
5.	1026.13 cm^{-1}	C – N	Stretch	Aliphatic amines
6.	864.11 cm^{-1}	C – H	Oop	Aromatic
7.	777.31 cm^{-1}	C – Cl	Stretch	Alkynes
8.	555.50 cm^{-1}	C –Br	Stretch	Alkyl halides



(2A)



(2B)

Fig. 2 (A) Colour change brown to green

(B) UV-VIS analysis of synthesized NPs shows peak at 270 nm

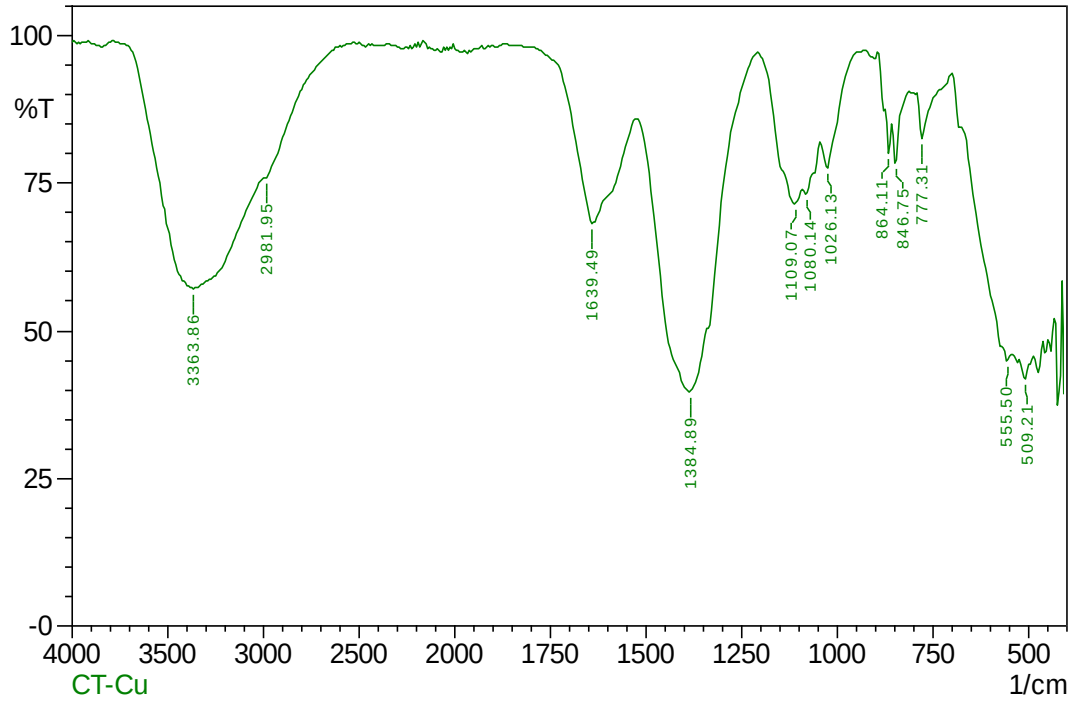


Fig. 3. FTIR spectra of green synthesized CuONPs from tuber extract of *C. tuberosa*

3.3 Particle Size and Zeta Potential Analysis of CuONPs

The particle size of CuONPs prepared from tuber extracts of *C. tuberosa* were analyzed by Dynamic Light Scattering experiment. The particle size distribution curve clearly showed that the particles have an average size of 14.5 nm (Fig. 4). Zeta potential analysis gives clear picture about the surface of nanoparticles and predicts the long-term stability of the nanoparticles formed. In the present study, the

copper oxide nanoparticles of *C. tuberosa* have showed Zeta potential of -20.2 mV (Fig. 5). The negative value indicates that the surface of nanoparticles is charged negatively. The negatively charged particles in the medium experience repulsion and hence exhibit stability. It is well known fact that the charge and size of the nanoparticles play important roles in the biological activity of prepared NPs.

3.4 X-RAY Diffraction Analysis of CuONPs of *C. tuberosa*

The XRD results of the synthesized Copper oxide nanoparticles of *C. tuberosa* spectrum shows distinct diffraction peaks at 2θ = 18.0^0, 27.8, 28.97^0, 32.14^0, 33.79^0, 36.66^0, 39.79^0, 39.87^0, 44.64^0, 52.38^0, 55.20^0, 56.240, 57.7^0 in the experimental diffractogram that have been identified due to copper metal and their corresponding *hkl* values (101), (112), (020), (200), (103), (113), (202), (004), (220), (204), (321), (303) and (321) respectively (Fig. 6) The above results confirmed that the resultant particles in the prepared sample are copper oxide nanoparticles having a face-centered cubic (fcc) crystal structure. The diffractograms have been compared with the standard powder diffractogram card of JCPDS silver file no. 89-3722.

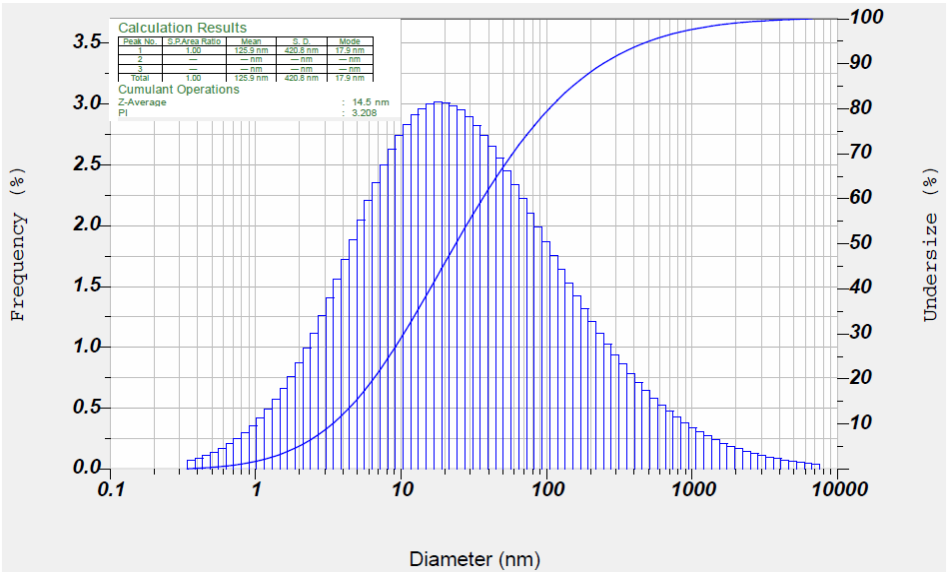


Fig. 4 Particle size distribution curve for *C. tuberosa* CuONPs

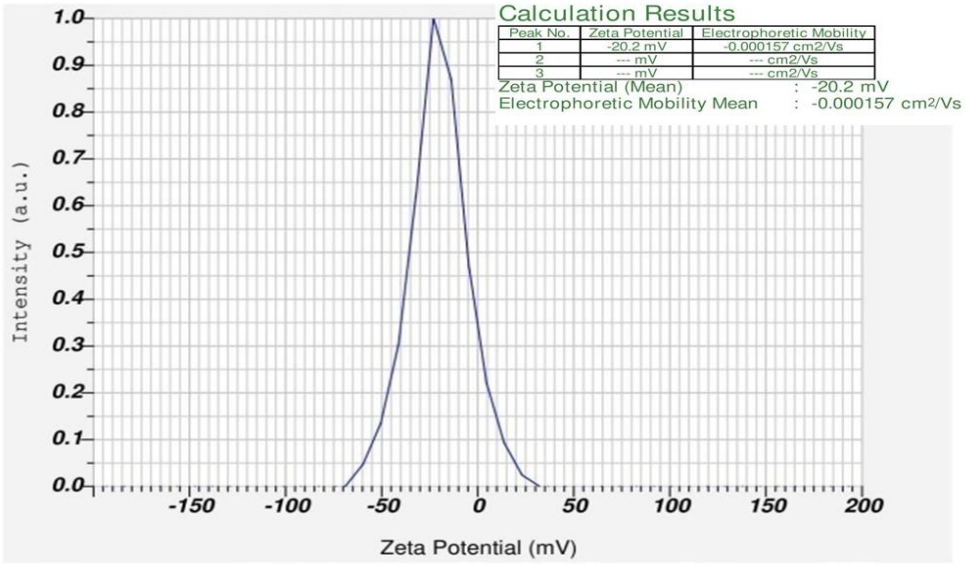


Fig. 5. Zeta potential of green synthesized CuONPs from tuber aqueous extract of *C. tuberosa*

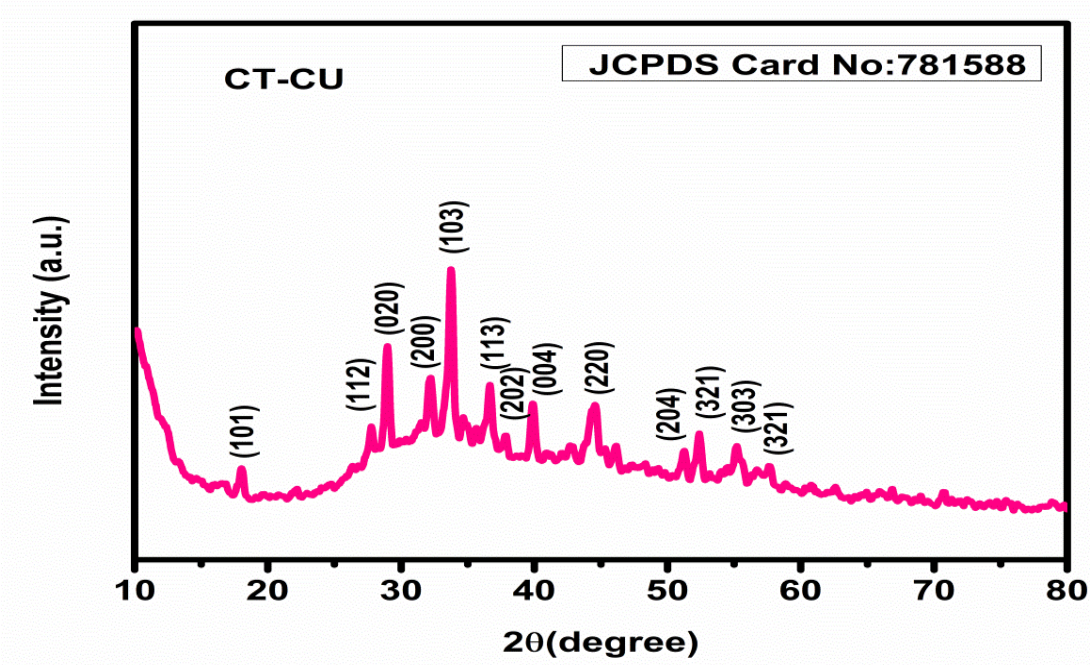


Fig. 6. XRD pattern of green synthesized NPs from tuber extract of *C. tuberosa*

3.5 High-Resolution Transmission Electron Microscopy (HR-TEM) Analysis

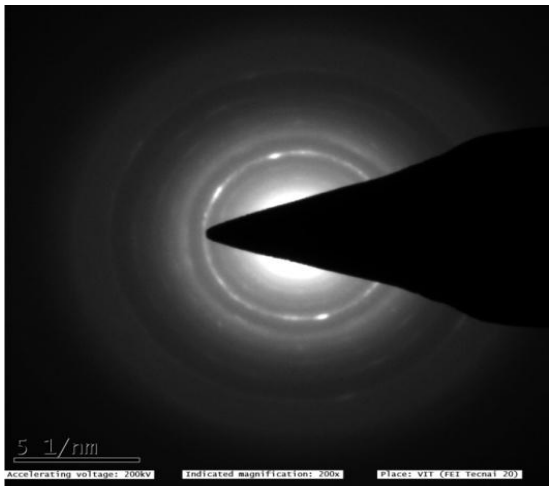
HR-TEM was used to analyze the morphology and size of the Copper oxide nanoparticles. The selected area of electron diffraction (SAED) shows clear diffused concentric rings (Fig. 7A), which are due to crystalline and polycrystalline spots. These results indicate that the synthesized nanoparticles were crystalline in nature. 20 nm scales bar TEM image (Fig. 7B) signified the synthesized nanoparticles with a size range from 1.52 to 3.31 nm. The 50 nm scale bar image (Fig. 7C) showed the particle size as 1.42 to 2.89 nm. The 100 nm scale bar image (Fig. 7D) showed the particles are spherical in shape.

3.6 EDX Spectrum of CuONPs of *C. tuberosa*

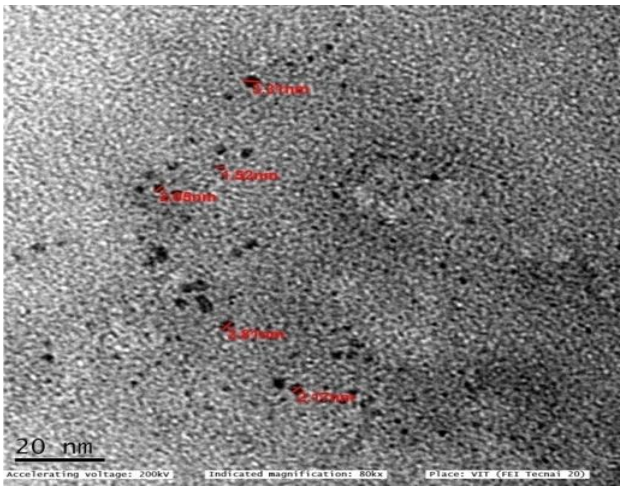
The experimental results of energy-dispersive spectrum of the synthesized CuONPs illustrate the presence of Cu as the ingredient element (Fig. 8). The elemental analysis of copper nanoparticles of *C. tuberosa* through EDX microanalysis revealed the presence of 49.38% weight percentage of copper metal along with 0.00% of carbon, 23.85% of sodium, 23.42% of oxygen, 0.92% of sulfur, 0.67% of chlorine, 1.04% of potassium and 0.72% of magnesium. The above results clearly show the abundance (49.38%) of copper in the preparation (Table 2).

3.7 Antibacterial Activity of Biosynthesized CuONPs from Aqueous Extract of *C. tuberosa*

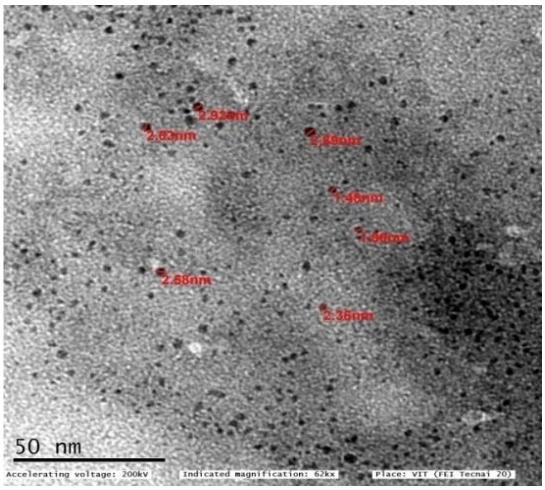
The green synthesized copper oxide nanoparticles of *C. tuberosa* were assessed for antimicrobial activities against two gram positive (*K. pneumonia* and *B. subtilis*) and two gram negative bacterial strains (*E. coli* and *S. aureus*). The aqueous extract of *C. tuberosa* and $CuSO_4.5H_2O$ solutions were used as controls. The antibiotic streptomycin was used as positive control for antibacterial activity. The antibacterial activity was measured by measuring the zone of inhibition formed in the agar plate. The zone of inhibition was observed in the order of *K. pneumonia* (15.25 mm) followed by *B. subtilis* (12.75 mm), *E. coli* (12.5 mm) and *S. aureus* (11.75 mm). The aqueous extract of *C. tuberosa* and $CuSO_4.5H_2O$ showed a limited zone of inhibition compared to *C. tuberosa* CuONPs (Figs. 9, 10 & Table 3). The above result clearly showed that the synthesized CuONPs have potent antibacterial activity against microbial pathogens compared to the aqueous extract of *C. tuberosa*. The results obtained with biosynthesized CuONPs are on par with the standard drug streptomycin.



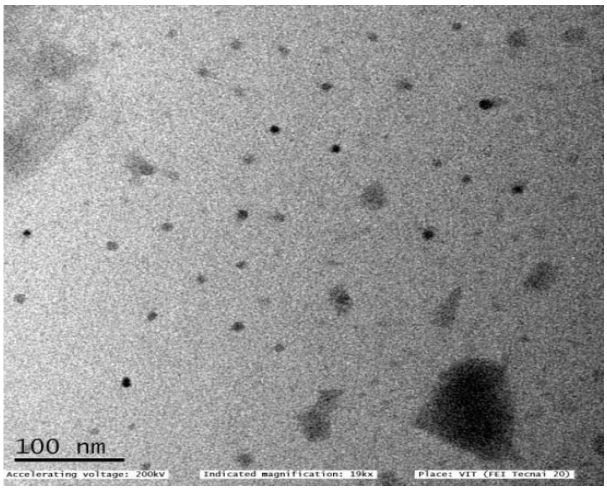
(7A)



(7B)



(7C)



(7D)

Fig. 7. (7A) Selected area electron diffraction (SAED) of green synthesized NPs, (7B) 20 nm resolution studies of green synthesized NPs shows mostly spherical shaped nanoparticles with 1.52 – 3.31 nm size. (7C) 50 nm resolution studies of green synthesized NPs nanoparticles with 1.42-2.89nm size. (7D) 100 nm resolution studies of green synthesized NPs shows mostly spherical shaped nanoparticles

Table 2. Spectrum: Spectrum 1091-CT-CuONPs

Elements	Series	Net	unn. C [wt. %]	corm. C [wt. %]	Atom. C [wt. %]	Error (3 sigma) [wt. %]
Sodium	K- series	47075	23.85	23.85	30.67	2.25
Carbon	K - series	0	0	0	0	0
Oxygen	K – series	25346	23.42	23.42	43.28	2.23
Copper	K – series	59885	49.38	49.38	22.97	4.57
Sulfur	K – series	1800	0.92	0.92	0.85	0.18
Chlorine	K – series	1279	0.67	0.67	0.56	0.16
Magnesium	K – series	1414	0.72	0.72	0.88	0.16
Potassium	K – series	2002	1.04	1.04	0.79	0.19
		Total	100.00	100.00	100.00	

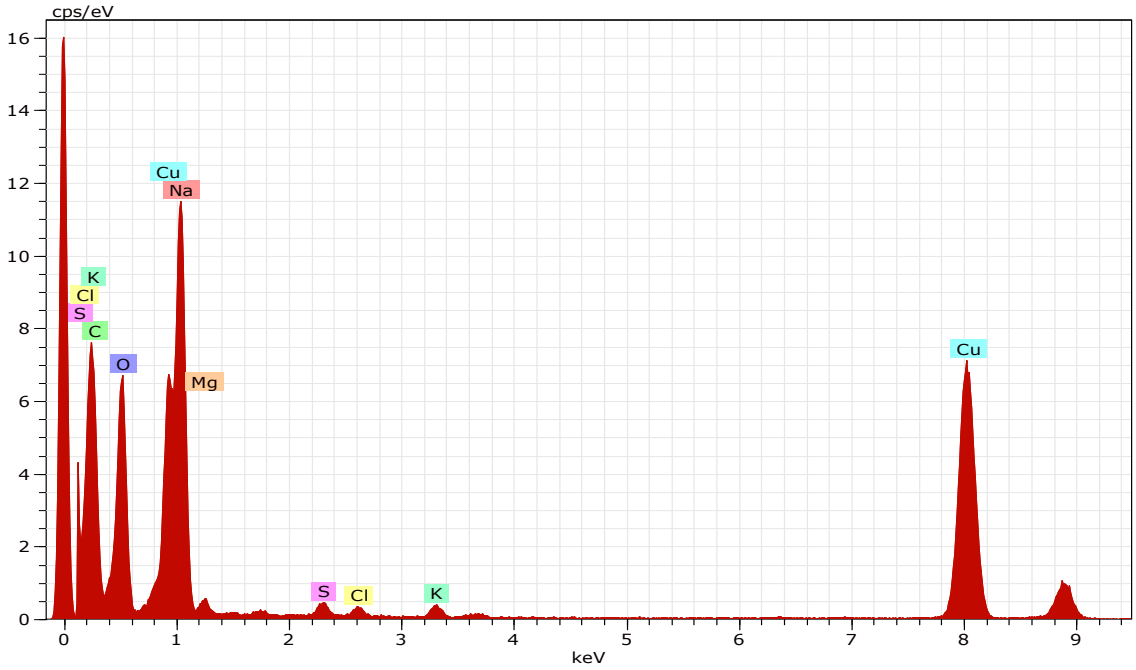
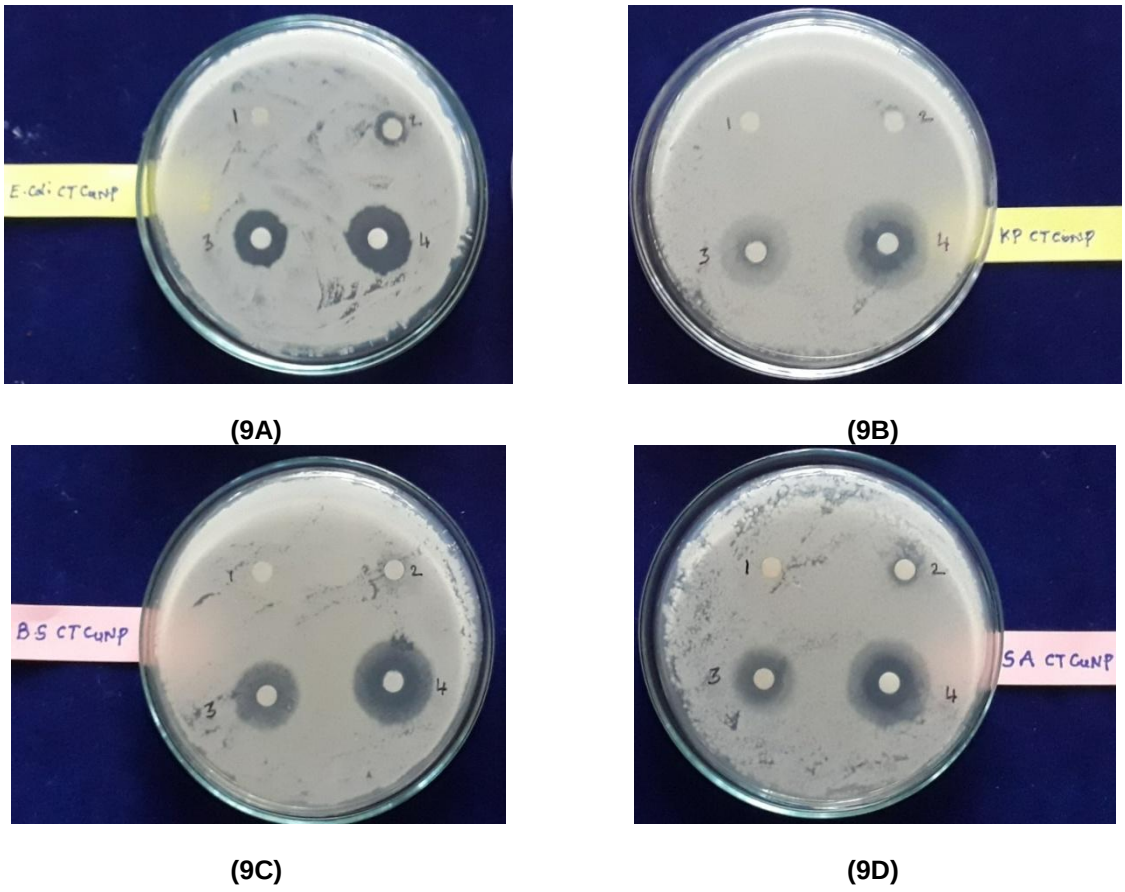


Fig. 8. EDX spectrum of green synthesized NPs from tuber extract of *C. tuberosa*



(9A) **(9B)**

(9C) **(9D)**

Fig. 9 Antimicrobial activities of CuONPs of *C. tuberosa* (9A) *E. coli*, (9B) *K. pneumoniae*, (9C) *B. subtilis*, (9D) *S. aureus*

Table 3. Effect of aqueous extracts and green synthesized copper oxide nanoparticles on clinically isolated bacterial strains

Name of Organism	Aqueous extract	$CuSO_4.5H_2O$	CuONPs	*Streptomycin*
E. coli	7 ± 0 ***	8 ± 0 ***	12.5 ± 0.65 **	15 ± 0.41
K. pneumoniae	7 ± 0.41 ***	7.5 ± 0.29 ***	15.25 ± 0.25 ***	17.75 ± 0.25
B. subtilis	6.75 ± 0.25 ***	6 ± 0 ***	12.75 ± 0.25 ***	16.5 ± 0.29
S. aureus	6 ± 0 ***	7 ± 0 ***	11.75 ± 0.25 ***	17.5 ± 0.29

*All the data are expressed as mean ±S EM: **p<0.01,* p<0.05 as compared to Control group, n=4: (One –way ANOVA followed by Dunnett's test)*

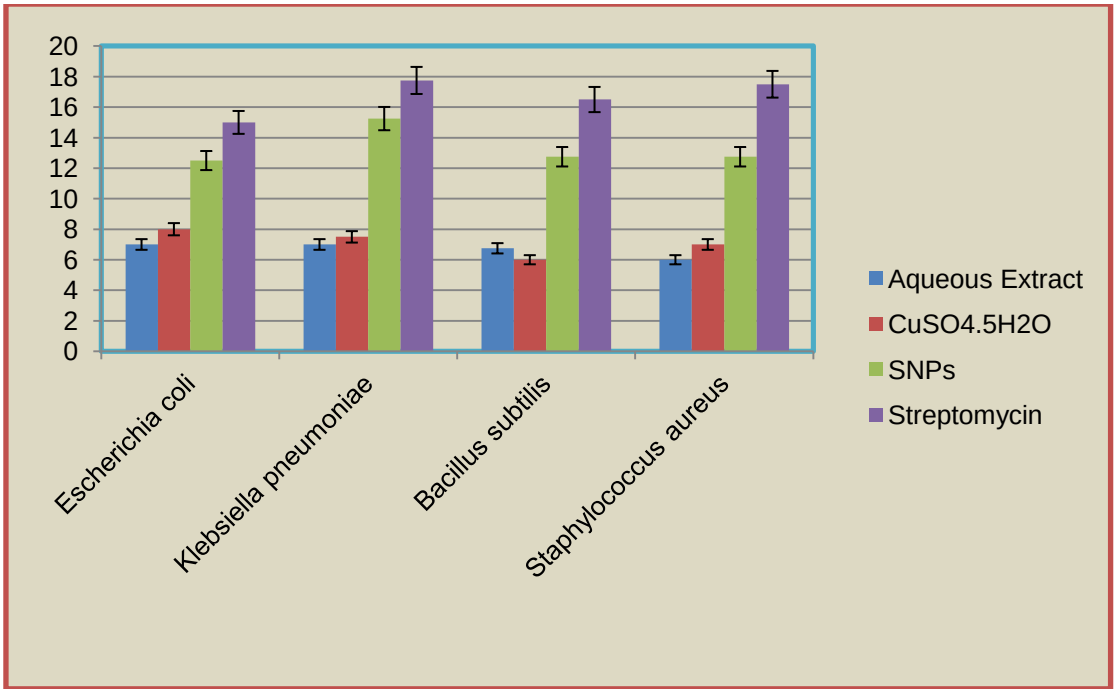
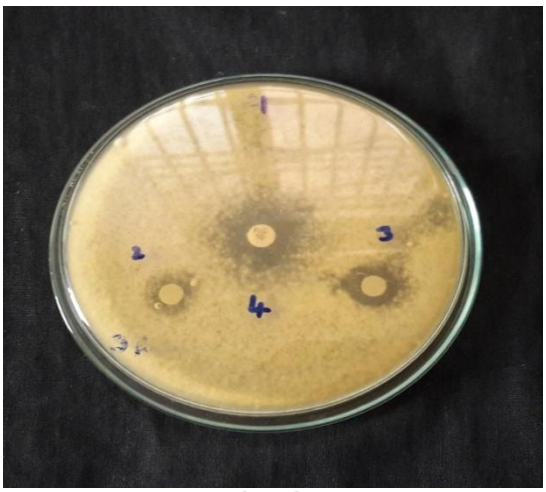
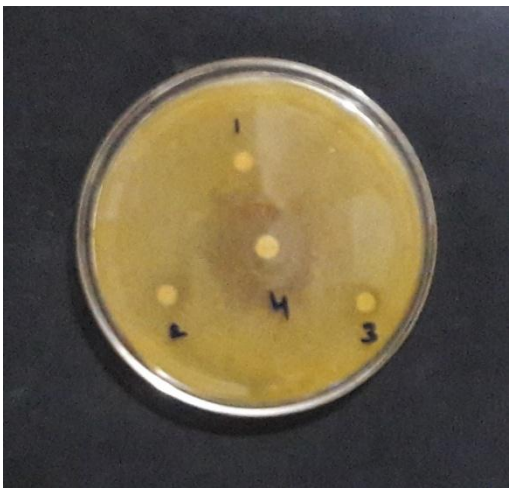


Fig. 10. Zone of inhibition of different extracts of *C. tuberosa* on clinically isolated bacteria



(11A)



(11B)

Fig. 11. Antifungal activity of biosynthesized CuONPs from aqueous extract of *C. tuberosa* (11A) *A. niger* (11B) *C. albicans*

Table 4. Comparison of different extracts of green synthesized Copper oxide nanoparticles effect on different clinical isolated fungi

Name of Organism	Aqueous Extract	$CuSO_4.5H_2O$	CuONPs	*Fluconazole*
A. niger	6.4 ± *0.04* ***	6.73 ± 0.11 ***	8.25 ± *0.03* ***	14.25 ± 0.63
C. albicans	6.38 ± 0.06 ***	7.43 ± *0.02* ***	9.45 ± 0.06 ***	19.75 ± 0.75

*All the data are expressed as mean ±S EM: **p<0.01,* p<0.05 as compared to control group, n=3: (One –way ANOVA followed by Dunnett's test)*

3.8 Antifungal Activity of Biosynthesized CuONPs from Aqueous Extract of *C. tuberosa*

The green synthesized copper oxide nanoparticles of *C. tuberosa* were assessed for antifungal activities against two fungal strains namely *C. albicans* and *A. niger*. The aqueous extract of *C. tuberosa* and $CuSO_4.5H_2O$ were also tested separately for antifungal activity. The antibiotic fluconazole was used as a positive control for antifungal activity. The antifungal activity was measured by measuring the zone of inhibition on plate. Among the two fungal strains the highest inhibition zone was observed on *C. albicans* (9.45 mm) followed by *A. niger* (8.25 mm). The aqueous extract of *C. tuberosa* and $CuSO_4.5H_2O$ showed a limited zone of inhibition compared to *C. tuberosa* CuONPs (Figs. 11, 12 & Table 4). The above result clearly showed that the synthesized CuONPs have potent antifungal activity against microbial pathogens. The results obtained with *C. tuberosa* CuONPs have nearest values with that of the standard drug fluconazole.

3.9 Antioxidant Activity of *C. tuberosa* Tubers Aqueous Extract

The antioxidant activity of aqueous tuber extract and CuONPs synthesized from tuber extracts of *C. tuberosa* were analyzed by DPPH radical scavenging assay. Ascorbic acid was used as a positive control for the assay. The experiment was performed with different concentrations of the test and standard drug. To measure the antioxidant potential, the lowest concentration was kept at 25 µg/ml where as the highest concentration was kept at 100 µg/ml. Both the samples namely aqueous tuber extract of *C. tuberosa* and the CuONPs prepared from extracts of *C. tuberosa* have shown the antioxidant activity. Both samples have shown the activity in a dose-dependent manner. These test samples activity was compared with the activity of the standard drug Ascorbic acid (Fig. 13). The highest antioxidant activity was shown by *C. tuberosa* CuONPs (56.32) followed by *C. tuberosa* aqueous tuber extract (49.34) at concentration 100 µg/ml. The standard drug Ascorbic acid (76.6) has shown the activity little higher tot that of *C. tuberosa* CuONPs (56.32) (Table 5). The above results clearly reveal that copper nanoparticles of *C. tuberosa* tuber extract possess good DPPH activity when compared to that of tuber extract alone. The antioxidant activity of CuONPs by the DPPH method shows a strong absorption band at 517 nm.

3.10 Anticancer Activity of CuONPs of *C. tuberosa* Tuber Extract

The CuONPs synthesized from *C. tuberosa* were tested for their anticancer activity by using MDA MB 453 (human breast cancer) cell line by MTT assay. The assay is a sensitive colorimetric method for the determination of number of viable cells in the cytotoxicity assays. In the present study, DMSO was used as negative control whereas Camptothecin was used as a positive control for anticancer activity or cytotoxicity. The cell lines were treated with the samples and incubated for 48 hours to see the result. During the treatment of cell lines, CuONPs were used in the concentration range of 12.5 µg/ml to 200 µg/ml. After incubation, a significant abatement in cell viability was observed in the CuONPs treated cell lines which are comparable to the positive control Camptothecin. The result clearly shows a decrease in proliferation rate with an increase in the concentration of CuONPs. DMSO, which was used as negative control exhibited 100% healthy proliferated cells (Fig. 14 & Table 6). The concentration that exhibits 50% reduction in the growth of tumor cells in culture is generally termed as half maximal inhibitory concentration (IC_{50}). CuONPs in the present study reduced the growth of MDA MB 453 cells by 50% at concentration 52.29 µg/ml. The cytotoxicity of nanoparticles may depend on the small size and spherical shape of the particles. Till now there is no report on CuONPs synthesized from *C. tuberosa* to attribute anticancer activity against MDA MB 453 (human breast cancer) cell lines. From this study, the green synthesized CuONPs from tuber extract exhibited strong cytotoxic activity against MDA MB 453 cell line.

Table 5. DPPH scavenging assay of green synthesized copper oxide nanoparticles

Content	Extracts	CuONPs	*Ascorbic acid*
25 µg/ml	11.44 ± 0.08	28.06 ± 0.18	46.36 ± 0.08
50 µg/ml	16.46 ± 0.16	34.13 ± 0.4	58.42 ± 0.6
75 µg/ml	28.32 ± 0.12	48.18 ± 0.16	68.16 ± 0.28
100 µg/ml	49.34 ± 0.24	56.32 ± 0.2	76.6 ± 0.62

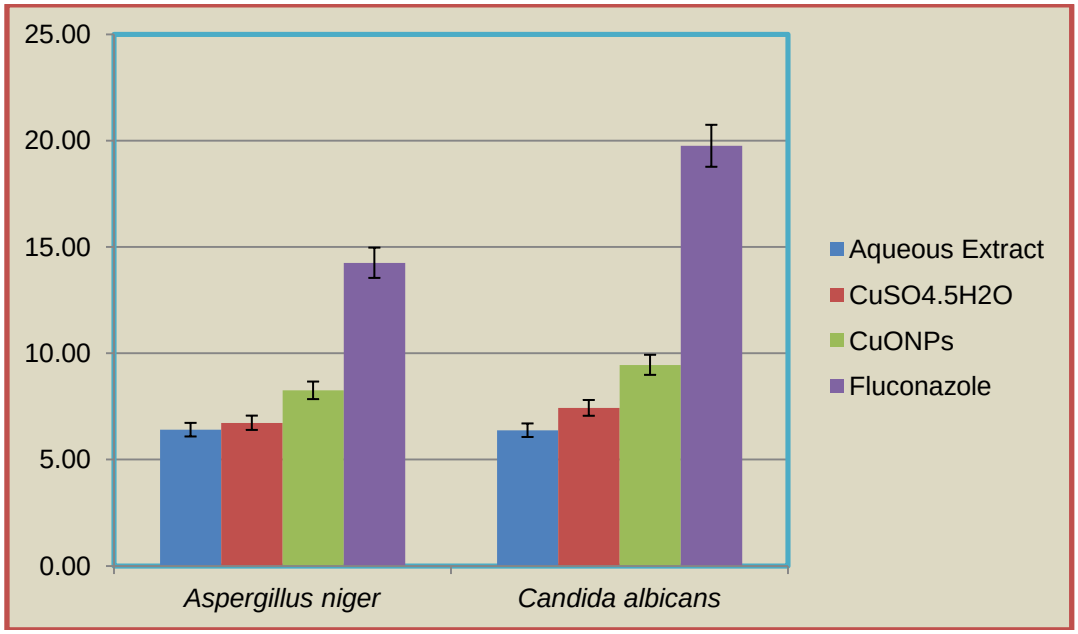


Fig. 12. Zone of inhibition of different extracts on clinically isolated fungi

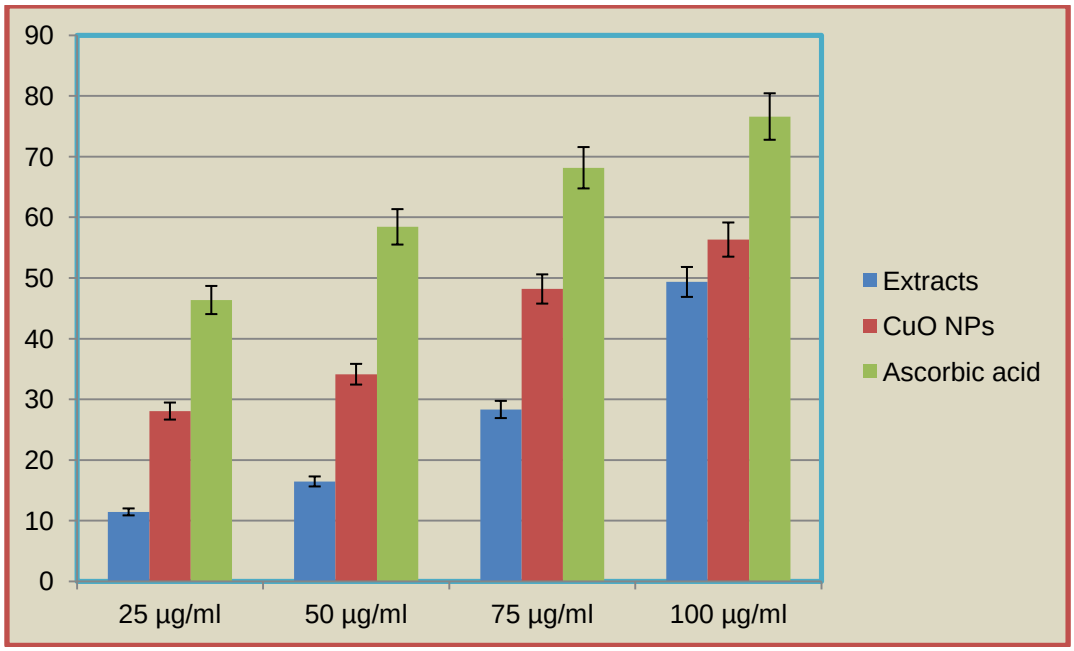
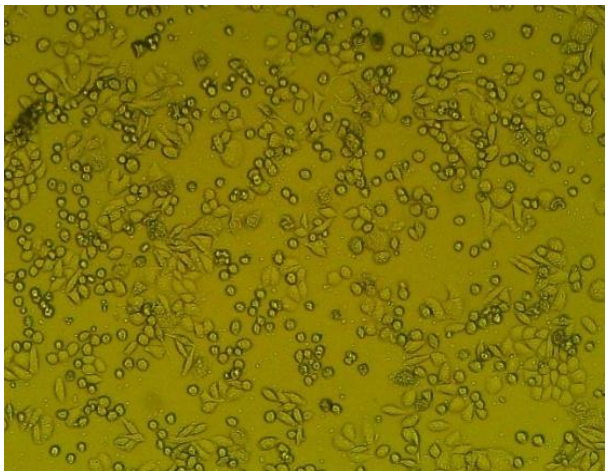
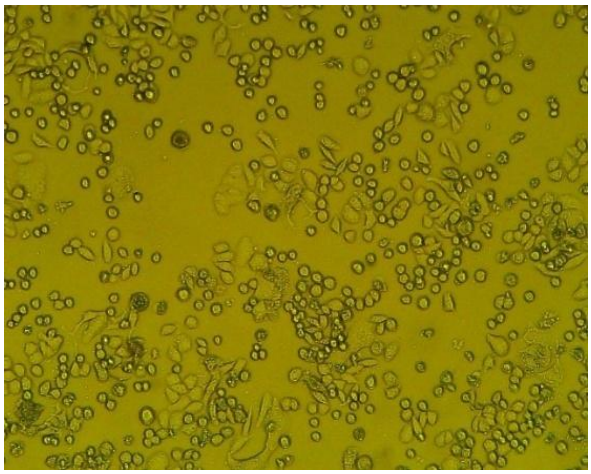


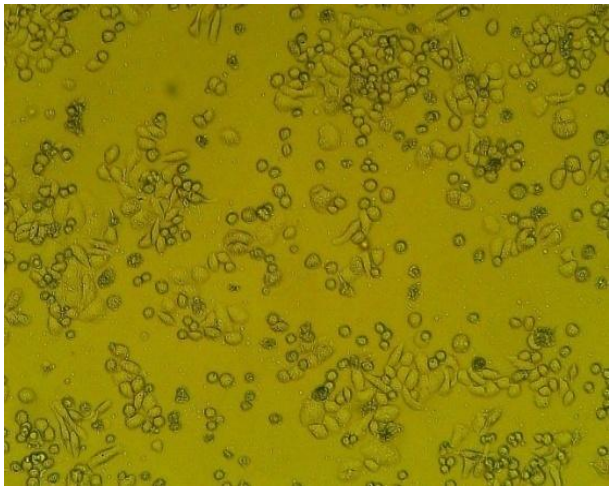
Fig. 13. DPPH scavenging assay of aqueous tuber extract of green synthesized CuONPs



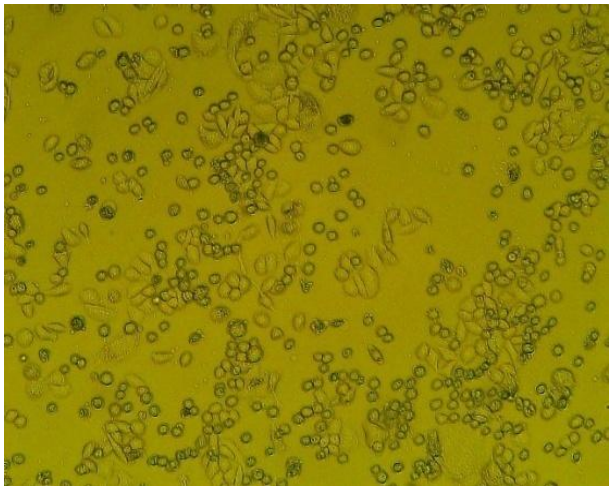
(14A)



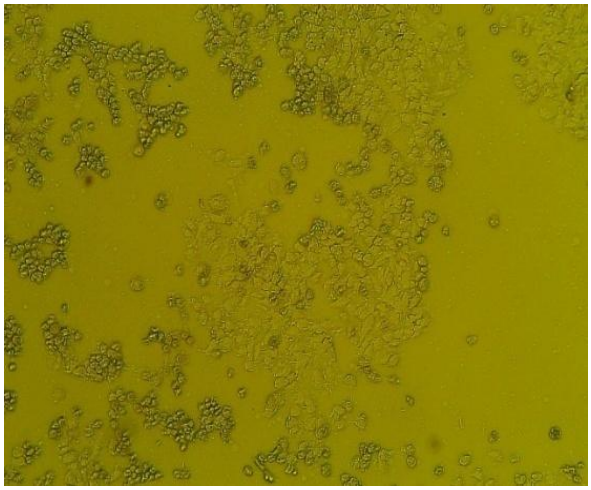
(14B)



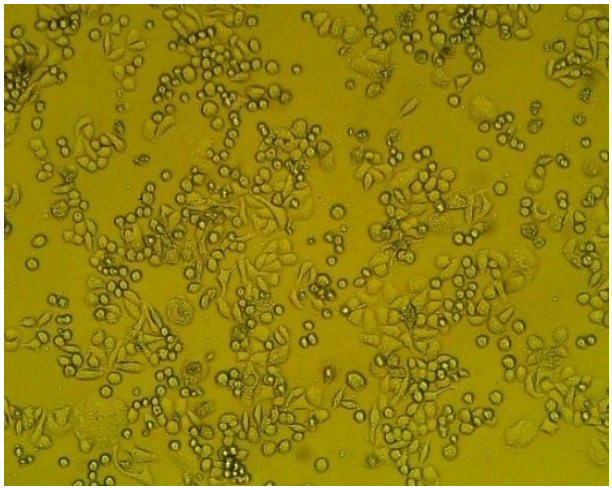
(14C)



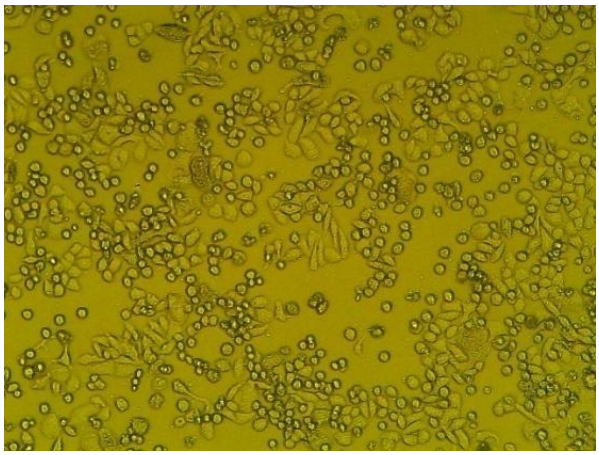
(14D)



(14E)



(14F)



(14G)

Fig.14. Anticancer activity of synthesized CuONPs 14A) 12.5µl/ml, 14B) 25 µg /ml, 14C) 50 µg/ml, 14D) 100 µg/ml, 14E) 200 µg/ml, 14F) DMSO, 14E) Camptothecin

Table 6. Dose dependent Cytotoxic activity of CuONPs

Concentration(µg/ml)	Absorbance (O.D)	Cell viability (%)	Cell Death (%)
DMSO	0.833	100	0
12.5	0.773	92.79	7.21
25	0.595	71.42	28.58
50	0.4285	51.44	48.56
100	0.2665	31.99	68.01
200	0.0665	17.98	82.02
Camptothecin	0.41	15.64	84.36

4. CONCLUSION

The present study was succeeded in synthesizing the copper oxide nanoparticles by using tubers aqueous extract of *C. tuberosa*. The plant has plenty full of several natural bioactive compounds that are capable of reducing copper ions and convert them to nano particles. The CuONPs that are synthesized were characterized in a sequential manner by employing various analytical techniques. The CuONPs thus characterized were tested for their antibacterial, antioxidant and anticancer properties. The present study clearly showed the antibacterial activity along with antioxidant and anticancer activity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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