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 *Tephrosia calophylla* rhizome Extract and Biological Evaluations.

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Abstract

In the present investigation, simple, robust and eco-friendly synthesis of copper oxide nanoparticles (CuONPs) using rhizome extract of *Tephrosia calophylla*. Biosynthesized *T.calophylla*-CuONPs was characterized by different spectroscopic techniques. UV–Vis spectrum showed the characteristic SPR peak 274 nm. EDX analysis revealed the presence of metallic copper at 3 keV. XRD analysis clearly revealed that CuONPs are crystalline in nature with FCC structure. TEM analysis depicted the spherical morphology with 5.97–9.32 nm in size. DLS analysis showed that average hydrodynamic size and PDI value of *T.calophylla*-CuONPs were found to be 129.2 nm and 10.06 respectively. Biosynthesized *T.calophylla*-CuONPs showed negative zeta potential value of − 21.7 mV. FTIR analysis revealed the participation of phenols and proteins in the bioreduction and stabilization of CuONPs. *T.calophylla*-CuONPs showed strong DPPH scavenging activity 50.2 % at 100 µg/mL. Antimicrobial studies of particles showed highest inhibitory activity against *Staphylococcus aureus* (14.5 mm) and on *Candida albicans* (16.25 mm) among bacterial and fungal strains, respectively. Further *T.calophylla*-CuONPs also showed effective cytotoxicity against cancer cell lines including MDAMB 453 (Human Breast Cancer) with maximum inhibition of 72.9% cell death and 27.1% 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.

KeyWords: Phenols, Bioreduction, Bacterial and fungal strains, Antioxidant activity, Antibacterial, Anticancer.

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 nanosize 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]. 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 CuO NPs 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.

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The medicinal plant *Tephrosia Calophylla* (Fig 1) was selected for the synthesis and charecterization of copper oxide Nanoparticles and their bioactivity through antibacterial sensitivity, antioxidant radical scavenging and cytotoxicity studies on human breast cancer cell lines. *T. calophylla* is a perennial rhizomatous sub shrub, mainly available in the Talakona forest area under shade. Leaves simple, parallel venation, Petiole winged, flowers light pink, pods compressed. Locally known as Adavi Vempali / Kommu vempali [15]. *Tephrosia Calophylla* used traditionally in folk medicine. According to Ayurveda, the plant is useful as an anti-helminthic, anti-pyretic and as well as an alexiteric drug. It is also active against leprosy, ulcers, cures diseases of the liver, spleen, heart and blood. According to the Unani system of medicine, the root is diuretic, allays thirst, enriches blood, cures diarrhea, it is also useful in bronchitis, inflammations, boils and pimples. Leaves are tonic to intestines, and a promising appetizer [16]. Hepatoprotective [17]: Antiplasmodial [18]: Anti cancer [19]: Anthelmintic activity [20]: Antiulcer activity [21]: Antimicrobial [22].

MATERIAL AND METHODS

Collection and Identification of Plant material:

T.calophylla plant material was collected from Talakona area, Chittoor District, Andhra Pradesh, India; Herbarium specimen was identified and deposited (Voucher No.GR:04) in the Department of Botany, Sri Venkateswara University, Tirupati [23]

Synthesis of CuONPs from *Tephrosia calophylla*:

T.calophylla Rhizome was 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 50^0C 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 *T.calophylla*

The green synthesized *T.calophylla* 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 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 [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 *T.calophylla* rhizome aqueous extract and *T.calophylla* 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].

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

Antimicrobial studies of SNPs

The antimicrobial activity of green synthesized copper oxide nanoparticles from *T. calophylla* rhizome extract was analyzed against two Gram positive bacterial strains like *Bacillus subtilis*(MTCC-441) , *Staphylococcus aureus*(MTCC-731) and Two Gram negative bacterial strains like *Escherichia coli* (MTCC-443), *Klebsiella pneumonia* (MTCC-741), as well as two fungal strains like *Aspergillus niger*(MTCC-281), *Candida albicans*(MTCC-183) by Disc diffusion method [33]. The antimicrobial activity with green synthesized CuONPs and comparative studies were made with plant rhizome extract as a positive control,1mM CuOSO4.5H2O as negative control and Streptomycin/Flucanozole (10mcg) as the standard. Sterile discs of 7 mm size were prepared from whatman No.1 filter paper and 20 µl of each extract was loaded on separate discs with the help of micro pipette and allowed to air dry for one hour in aseptic conditions. Freshly prepared nutrient agar media for bacterial culture substrate was poured into sterile Petriplates and allowed 30 minutes for solidification. The plates were swabbed with microbial cultures and placed the previously prepared discs; the experiment was carried out in triplicates. The plates were incubated at 37 °C for 24 to 48 h then the zone of inhibition was measured.

Anticancer activity

CuONPs of *T.calophylla* 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 570nm [34-35].MDAMB 453 cell line (Human Breast Cancer) 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 2×10^4 cells per well in 96-well plates and incubated in 5% CO_2 atmosphere at 37^0C 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 *T.calophylla incubated* for 24hrs. Further, remove spent media and add 100 µl 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 100ul 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 37^0c incubation period. The percentage of cell viability was calculated by the following formula [37]

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

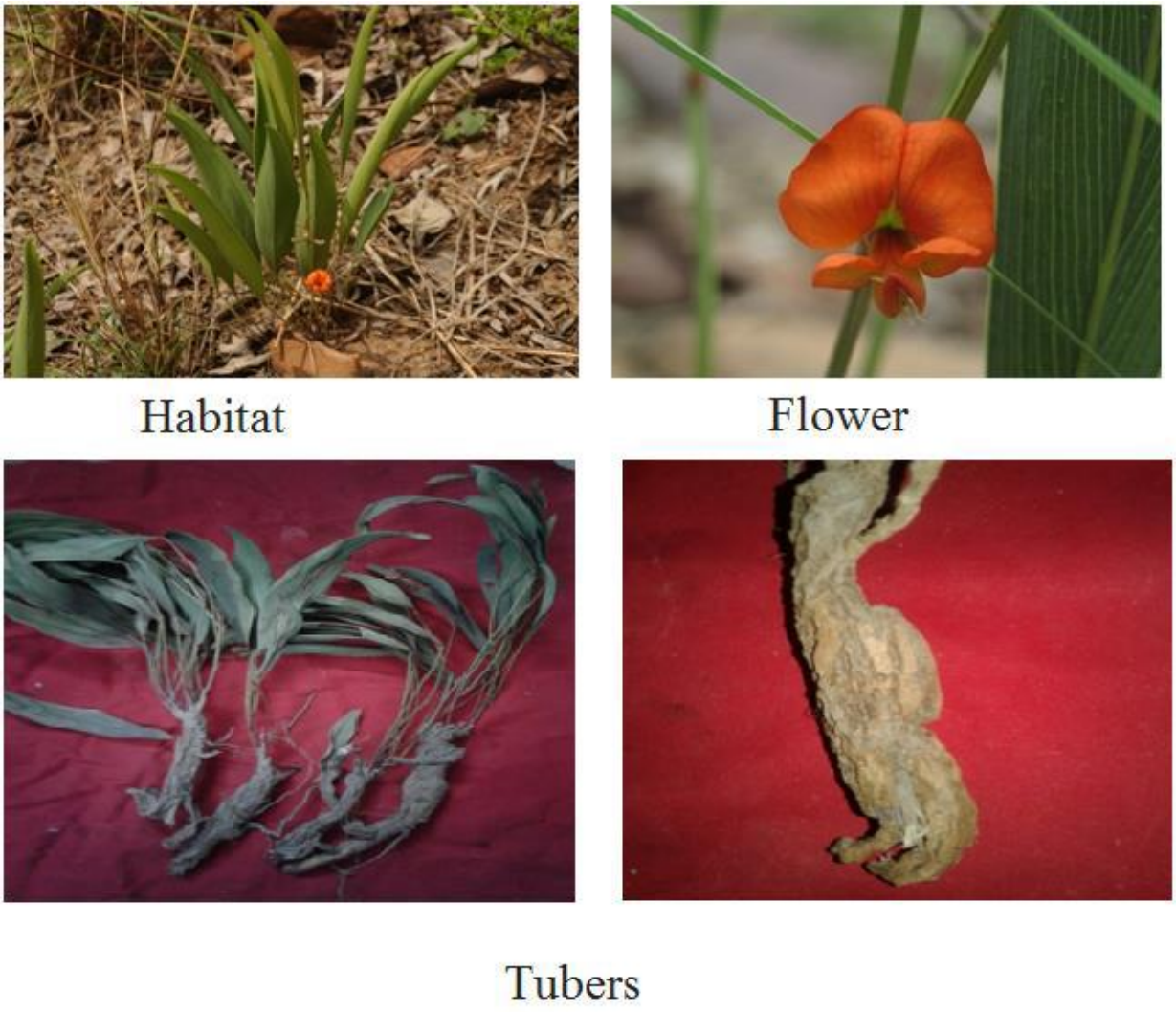


Fig.1 ***Tephrosia calophylla***

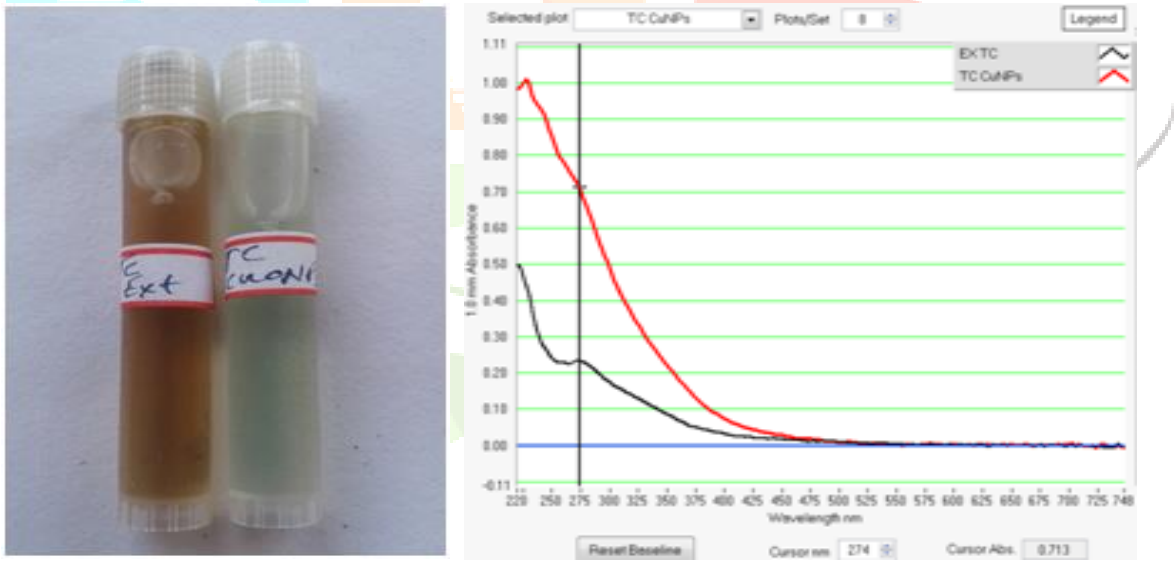
RESULTS

Ultra violet–visible spectroscopy of *T. calophylla* -CuONPs:

It is observed that rhizome extract of ***T.calophylla*** mediated CuONPs manifest a color change from **brown to light green** (**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 1 hrs.

After the reduction of CuO material, they formed as spherical shaped CuO nanoparticles by the action of plant phytochemicals. Which as 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. The nanoparticles in the reaction mixture were excited by absorbing light at different wavelengths due to surface plasmon resonance (SPR) nature to give respective peak represents the particular metal nanoparticles were actually reduced. Synthesized reaction mixture showed that a peak at **274 nm** corresponds to copper oxide, which confirms the synthesized nanoparticles were CuONPs.

UV-VIS ABSORPTION SPECTRA of green synthesized CuONPs



(a) **(b)**

Fig 2: (a) Colour change brown to light green. (b) UV-VIS analysis of synthesized SNPs shows peak at 274 nm.

FTIR spectra of green synthesized CuONPs from Rhizome extract of *T. calophylla.*

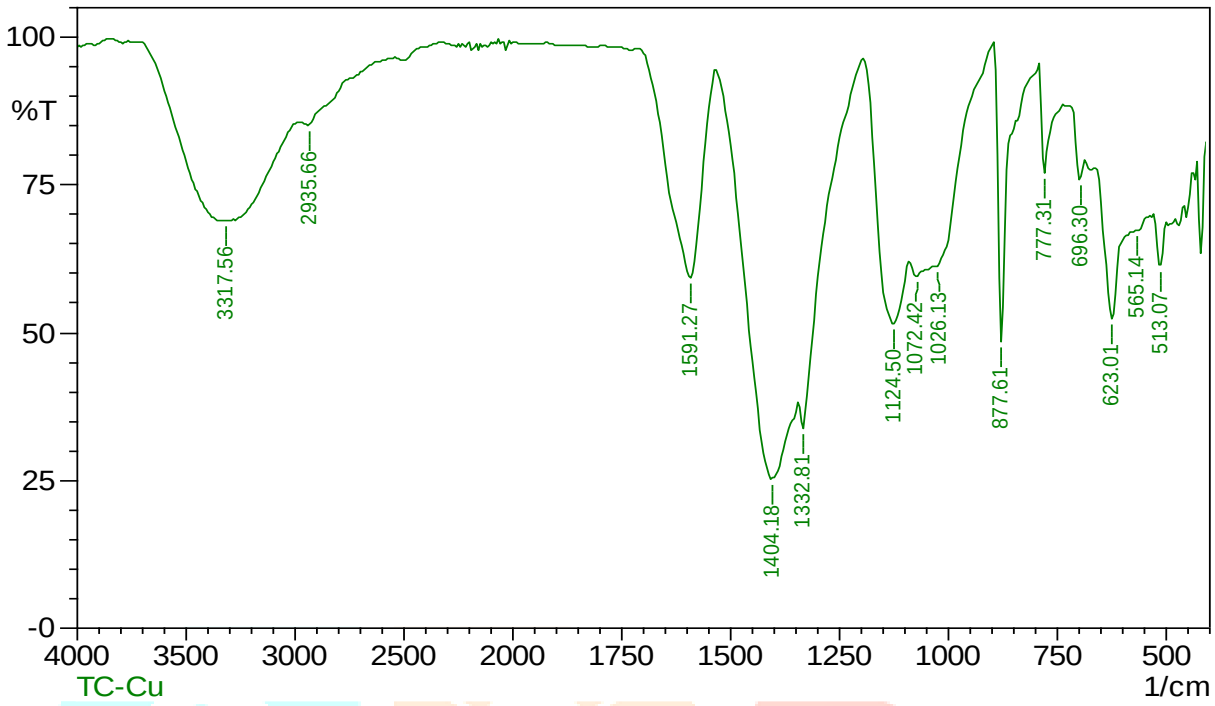


Fig.3 FTIR spectra of green synthesized CuONPs from rhizome extract of *T.calophylla*

In FTIR analysis of aqueous Rhizome extract of *T. calophylla*, the peaks observed at **3317.56 cm^{-1}** for O – H (Stretch) bond of Phenols, **2935.66 cm^{-1}** for N – H (Bend) bond of amines, **1591.27 cm^{-1}** for C – C (Stretch) bond of aromatic,**1404.18 cm^{-1}** for C - H (Bend) bond of alkenes, **1332.81 cm^{-1}** for N – O (symmetric stretch) bond of Nitro compounds, **1124.50 cm^{-1}** for C – N (Stretch) bond of aliphatic amines, **1072.42 cm^{-1}** for C – H (Wag) bond of alkyl halides, **1026.13 cm^{-1}** for C – O (Stretch) bond of alcohol, **877.61 cm^{-1}** for C – H (Bend) bond of alkanes, **777.31 cm^{-1}** for N – H (Wag) bond of amines, **696.30 cm^{-1}** for C – Cl (Stretch) bond of alkyl halides, **623.01 cm^{-1}** for C – CH (rock) bond of alkanes, **565.14 cm^{-1}** for C – Br (Stretch) bond of alkyl halides **(Fig.3)**. The results reveal that different phyto-constituents like carbohydrates, starch, tannins, flavonoids, and polyphenols of the rhizome extract have been actively participated in reduction of Copper oxide to *T.calophylla* -CuONPs, in capping and in stabilization of the nanoparticles. Bioactive compounds consist different functional compounds which are reduced in the process of biosynthesis of *T.calophylla*-CuONPs.

Particle size and Zeta potential analysis of *T.calophylla* - CuONPs:

The particle size of the biosynthesized ***T.calophylla* - CuONPs** is detected by the intensity and laser diffraction method using the biosynthesized colloidal solution in which the ***T.calophylla* - CuONPs** are polydispersed in mixture solution. The distribution of ***T.calophylla*** - average size was found to be **129.2 nm** (**Figure 4 a & b)** with PI value of **10.063** (Polydispersity index). Further the zeta potential analysis of ***T.calophylla*** CuONPs were detected to be **-21.7 mV**, due to its high negative zeta potential it prevent the

T.calophylla CuONPs from agglomeration in the medium, leading to long term stability, because of the electrostatic repulsive force.

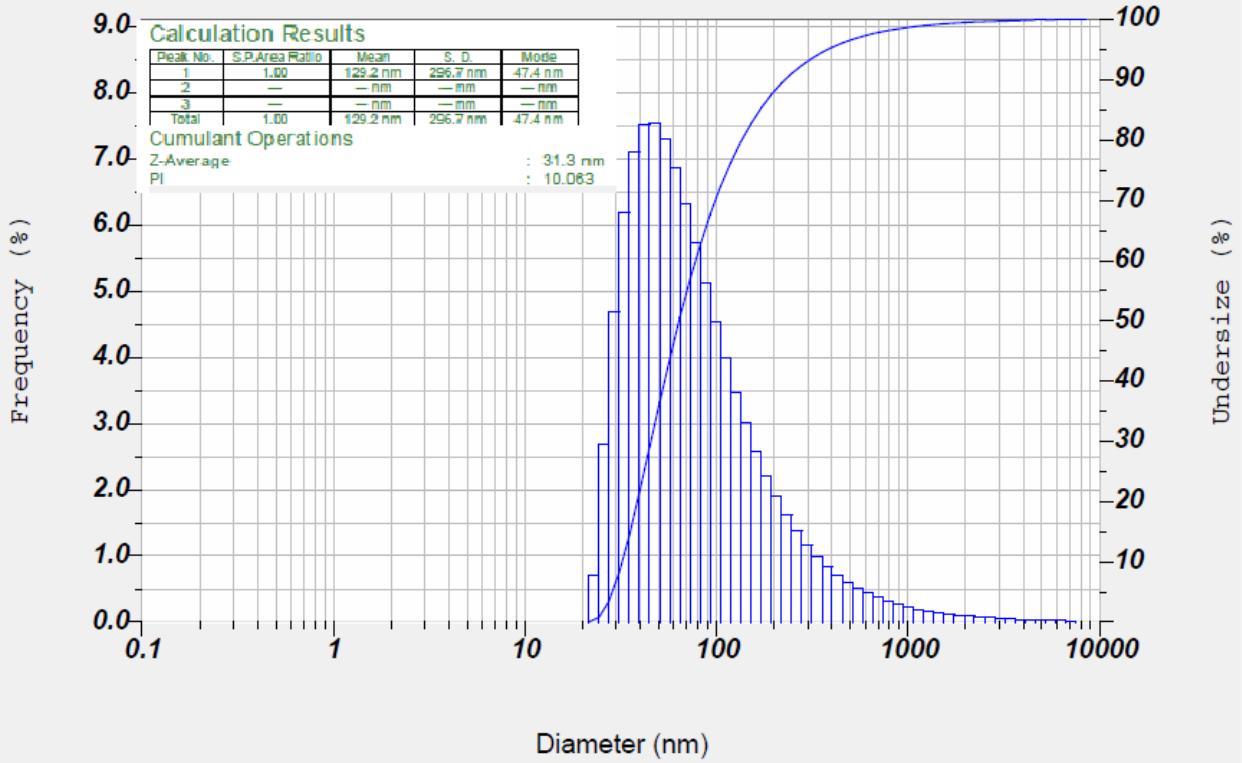


Fig 4. (a) Particle size distribution curve for ***T. calophylla***– CuONPs.

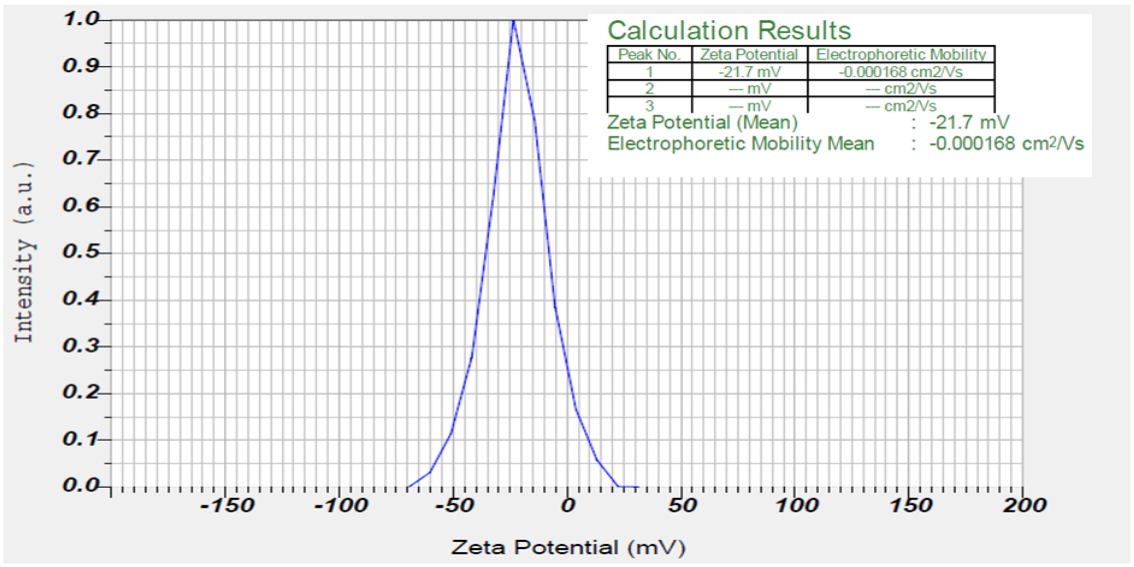


Fig.4 (b) Zeta potential of green synthesized CuONPs from Rhizome extract of ***T. calophylla***.

X-RAY Diffraction Analysis for *T. calophylla*: CuONPs

The XRD results of the synthesized Copper oxide nanoparticles of ***T. calophylla*** spectrum shows distinct diffraction peaks at $2\theta = 15.18^0$, 24.06^0, 31.70^0, 33.60^0, 34.81^0, 41.68^0, 44.68^0, 49.35^0 have been identified due to copper metal and corresponding to *hkl* values (010), (012), (200), (103), (022), (214), (220) and (131) respectively (**Fig.5)**. Thus, 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 file no. 781588,710251.

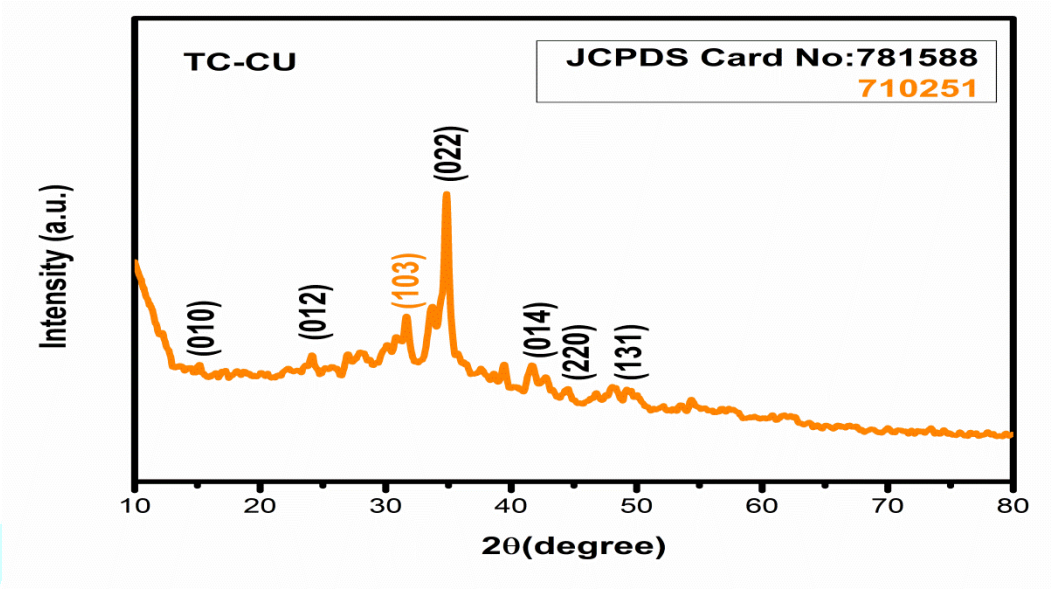


Fig:5 XRD pattern of green synthesized SNPs from Rhizome extract of ***T. calophylla***.

Transmission electron microscopy (TEM) analysis of *T.calophylla* – CuONPs

TEM was used to analyze the morphology and size of the Copper oxide nanoparticles. The selected area of electron diffraction (SAED) of *T. calophylla* CuONPs shows clear diffused concentric rings **(Fig 6)** which are due to crystalline and polycrystalline spots. The HR-TEM image of biosynthesized copper oxide nanoparticles showing the lattice fringes quite clearly like projections of tunnels. 100-nm scale bar studies of TEM micrographs of CuONPs signify that the synthesized nanoparticles are polydispersed, spherical shaped nanoparticles between 5.97-9.32 nm size and are not in physical contact with each other i.e., no agglomeration of nanoparticles were seen.

The energy-dispersive spectrum of the synthesized CuONPs, which illustrates the presence of Cu as the ingredient element. The quantitative information of biosynthesized CuONPs of *T. calophylla* EDAX analysis demonstrated the presence of **16.34%** weight percentage of copper metal along with **64.37%** carbon, **4.49%** Oxygen,**1.74%** Silicon, **2.45%** Chlorine, **8.94%** Iron **(fig 7) .** Indicates the sample having high purity of copper nanoparticles.

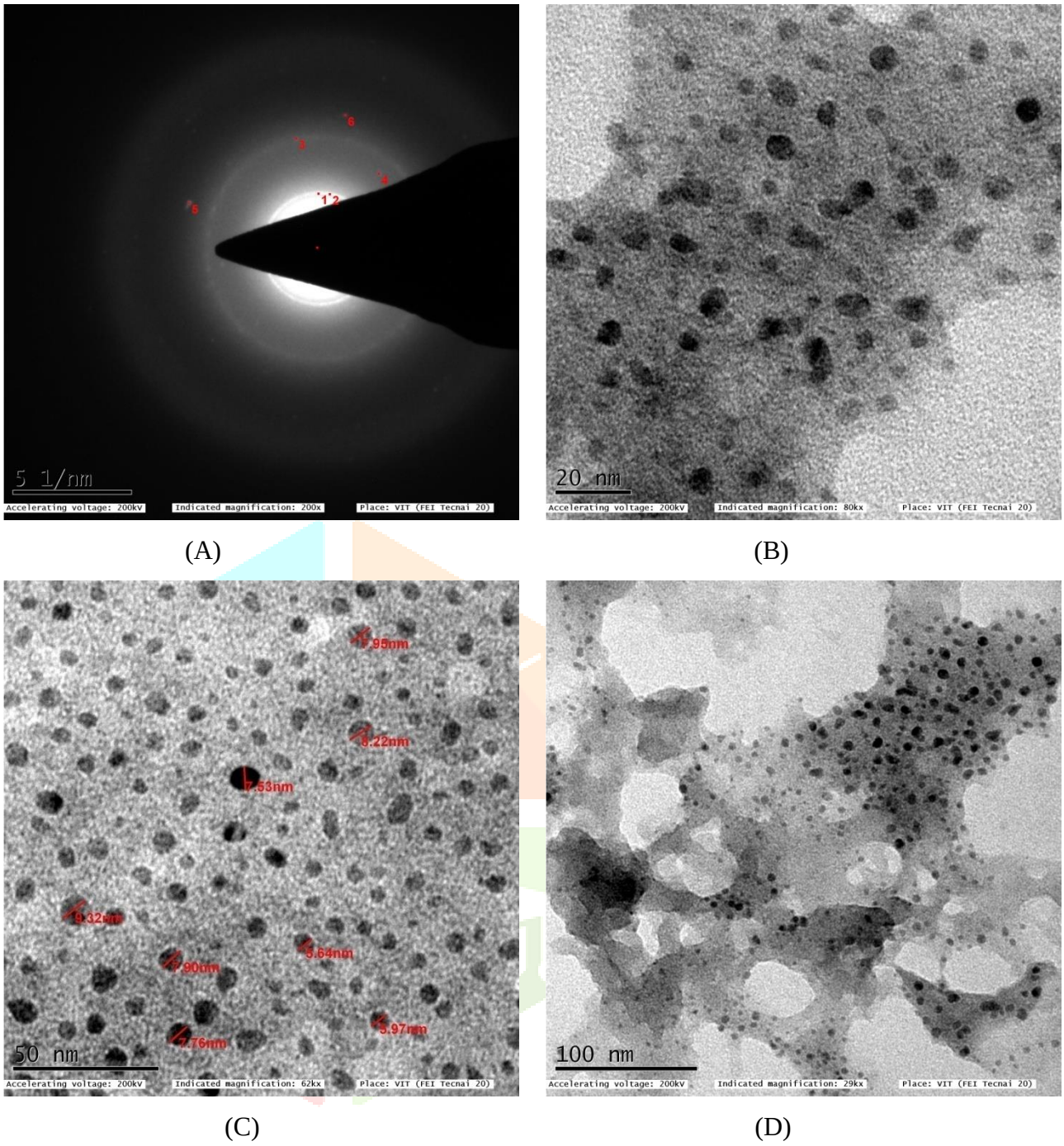


Fig 6 :(A) Selected area electron diffraction (SAED) of green synthesized SNPs, (B) 20 nm resolution studies of green synthesized SNPs. (C) 50 nm SNPs with 5.97 - 9.32 nm size. (D) 100 nm SNPs shows mostly spherical shaped nanoparticles.

EDX SPECTURM of biosynthesized *T. calophylla* – CuONPs analysis:

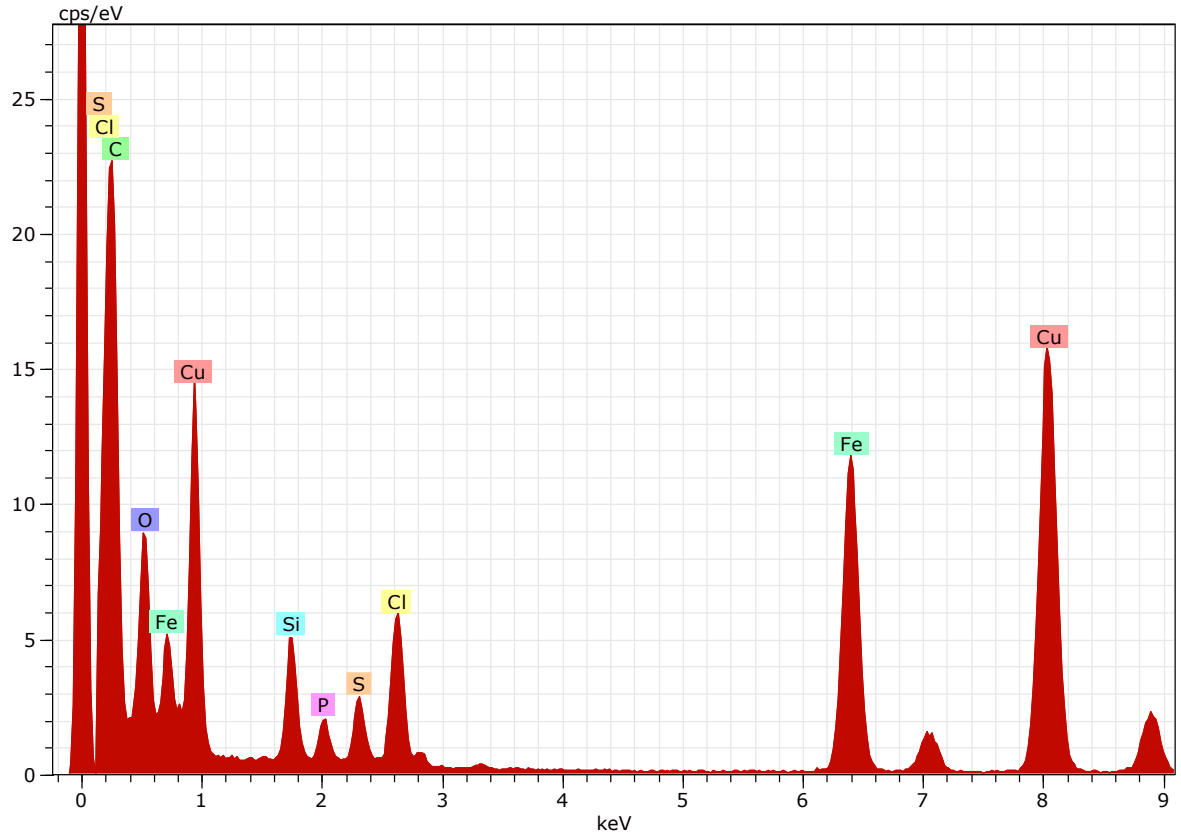


Fig 7: EDX analyses of green Synthesized CuONPs shows 16.34 weight percentages.

Spectrum: Spectrum 1486

Element	Series	Net unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Copper	K-series	48106	16.34	4.12	1.56
Carbon	K-series	24153	64.37	85.87	6.00
Oxygen	K-series	11796	4.49	4.50	0.50
Silicon	K-series	8097	1.74	0.99	0.13
Phosphorus	K-series	3095	0.68	0.35	0.15
Chlorine	K-series	11360	2.45	1.11	0.31
Sulfur	K-series	4707	0.99	0.49	0.17
Iron	K-series	32465	8.94	2.56	0.89
	Total:		100.00	100.00	

Antioxidant activity of biosynthesized Copper oxide nanoparticles of *T. calophylla.*

The aqueous rhizome extract and synthesized CuONPs of ***T. calophylla*** showed better antioxidant potential when compare to standard ascorbic acid by DPPH scavenging assay method. Different concentrations ranging between 25-100 µg/ml of ***T. calophylla* rhizome** aqueous extract and ***T. calophylla* CuONPs**; Ascorbic acid was taken as a positive control to compare the percentage activity of the aqueous rhizome extract and copper oxide nanoparticles **(Fig 8 & Table 1).** The antioxidant activity was increased in dose-dependent manner. The highest percentage activity was exhibited at 100 µg/ml ***T. calophylla* rhizome**

extract (33.4) < *T. calophylla* CuONPs (50.2) < Ascorbic acid (76.6). It is concluded that copper oxide nanoparticles of *T. calophylla* possess good DPPH activity when compared to that of rhizome extract alone. The antioxidant activity of CuONPs by the DPPH method shows a strong absorption band at 517 nm.

Table 1 : Antioxidant activity of biosynthesized *T.calophylla* - CuONPs

Content	Extracts	CuONPs	Ascorbic acid
25 µg/ml	12.02 ± 0.16	12.42 ± 0.24	46.36 ± 0.08
50 µg/ml	17.14 ± 0.15	15.21 ± 1	58.42 ± 0.06
75 µg/ml	25.02 ± 0.1	35.14 ± 0.18	68.16 ± 0.28
100 µg/ml	33.4 ± 0.16	50.2 ± 0.26	76.6 ± 0.62

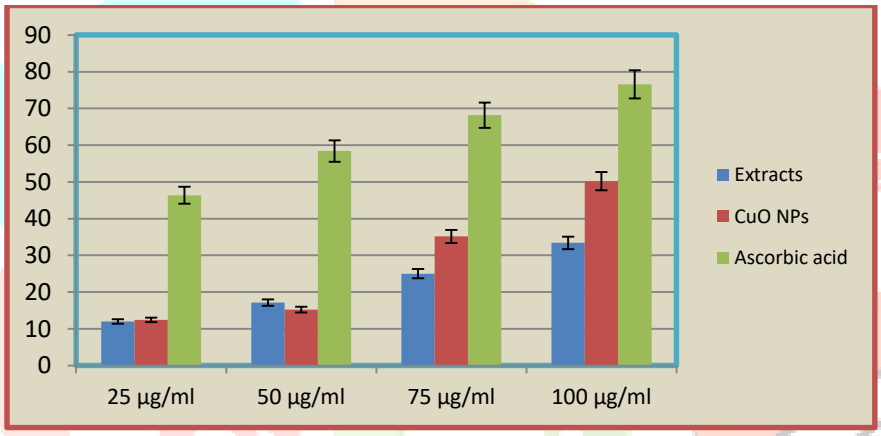


Figure 8: Antioxidant activity of biosynthesized ***T.calophylla***– CuONPs

Antibacterial activity of biosynthesized CuONPs from aqueous rhizome extract of *T. calophylla:*

The green synthesized copper oxide nanoparticles of ***T. calophylla*** were assessed for antimicrobial activities against two gram positive and two gram negative bacterial strains. Among the bacteria the highest Inhibition zones were observed on ***Staphylococcus aureus* (14.5 mm)** followed by ***Klebsiella pneumoniae* (14 mm), *Escherichia coli* (13.75 mm)** and ***Bacillus subtilis* (10.5 mm)**. The aqueous extract of ***T. calophylla*** and CuSO4.5H2O showed a limited zone of inhibition compared to ***Tc*-CuONPs (Figs 9&10, Tables 2)** .The above result clearly showed that the synthesized CuONPs have potent antibacterial activity against microbial pathogens.

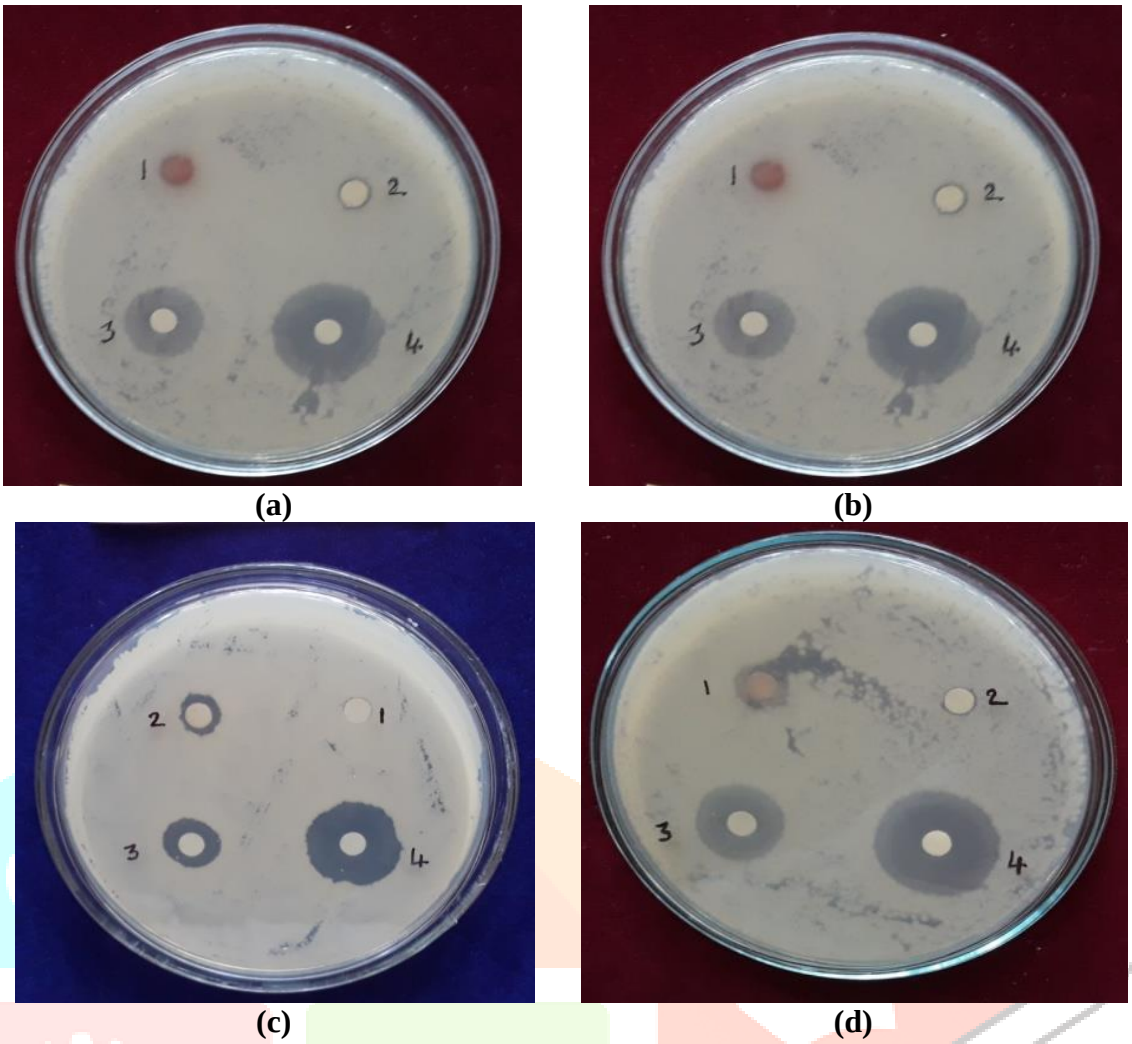


Fig 9: **(a)** *Escherichia coli*, **(b)** *Klebsiella pneumonia*, **(c)** *Bacillus subtilis* and **(d)** *Staphylococcus aureus*

Table 2: Effect of different extracts and green synthesized copper oxide nanoparticles on clinically isolated bacterial Strains.

Name of Organism	Aqueous Extract	CuSO4.5H2O	CuONPs	Streptomycin
Escherichia coli	8.25 ± 0.25 ***	7 ± 0.41 ***	13.75 ± 0.25 ***	19.25 ± 0.75
Klebsiella pneumoniae	6.5 ± 0.29 ***	6 ± 0 ***	14 ± 0 ***	18.75 ± 0.25
Bacillus subtilis	7.25 ± 0.25 ***	6 ± 0 ***	10.5 ± 0.29 ***	15.25 ± 0.25
Staphylococcus aureus	6.25 ± 0.25 ***	7 ± 0 ***	14.5 ± 0.29 ***	18.25 ± 0.48

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).

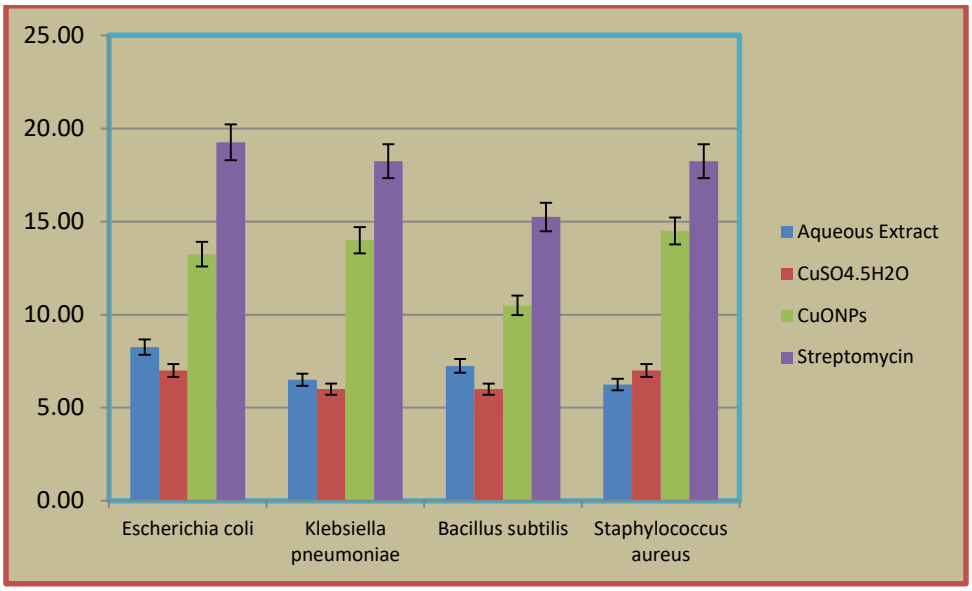


Fig10 : Zone of inhibition of different extracts on clinically isolated bacterial strains

Antifungal activity of biosynthesized CuONPs from aqueous extract of *T. calophylla:*

The green synthesized copper oxide nanoparticles of *T. calophylla* were assessed for antifungal activities against two fungal strains. Among the two fungal strains the highest Inhibition zones were observed on *Candida albicans* (16.25 mm) followed by *Aspergillus niger* (14.25 mm) **(Fig.11&12 &Tables 3)**. The above result clearly showed that the synthesized CuONPs have potent antifungal activity.

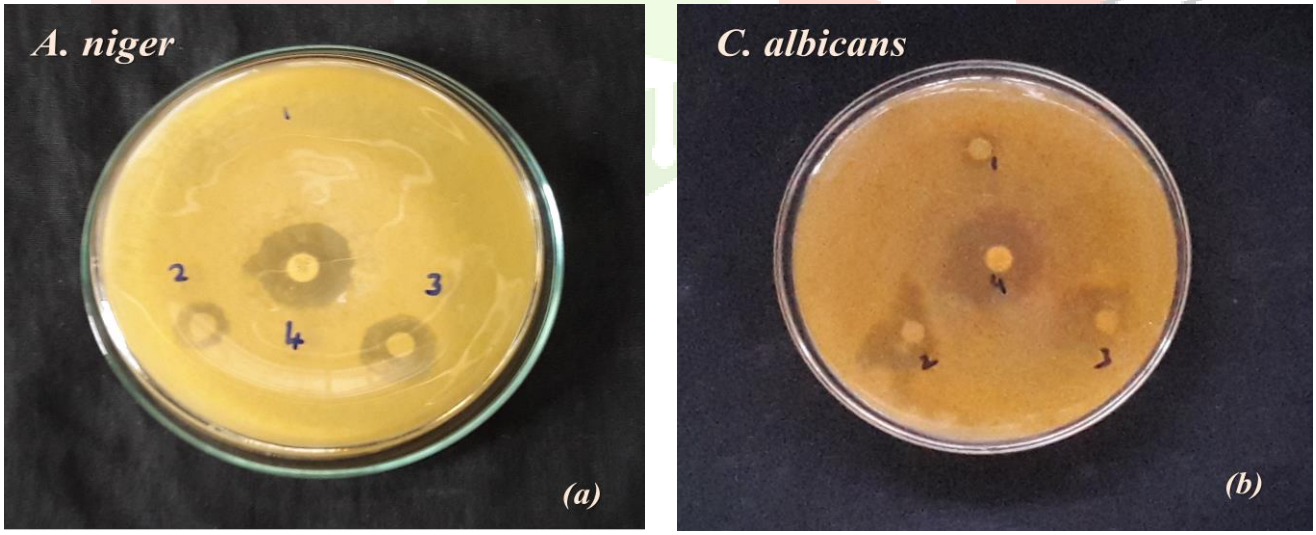


Fig 11 Antifungal activity of biosynthesized CuONPs from aqueous extract of *T.calophylla*

Table 3: Comparison of different extracts of green synthesized CuoNPs of *T.calophylla* nanoparticles effect on different clinical isolated fungi.

Name of Organism	Aqueous Extract	CuSO4.5H2O	CuONPs	Fluconazole
Aspergillus niger	6.40 ± 0.08 ***	7.38 ± 0.09 ***	14.25 ± 0.63 ***	20 ± 0.41
Candida albicans	6.33 ± 0.05 ***	8.10 ±0.04 ***	16.25 ± 0.63 ***	21.25 ± 0.48

Values of average of triplicates. ; ± S.E

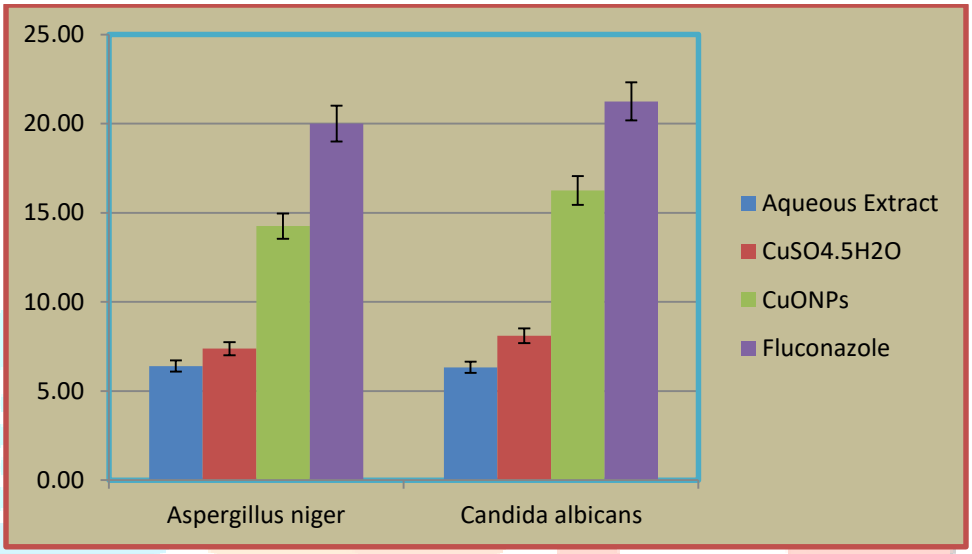
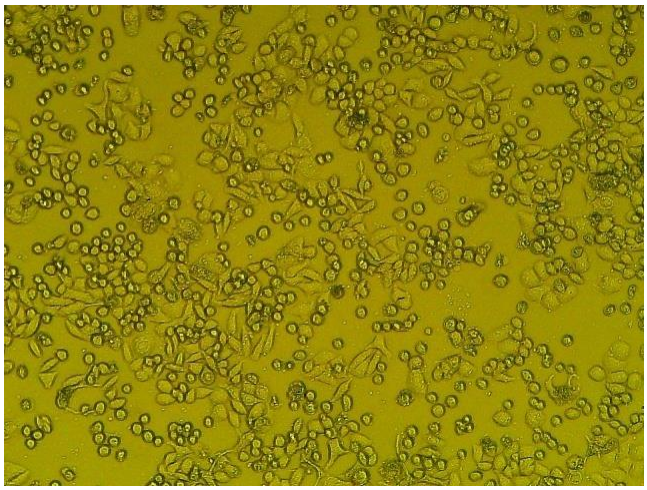


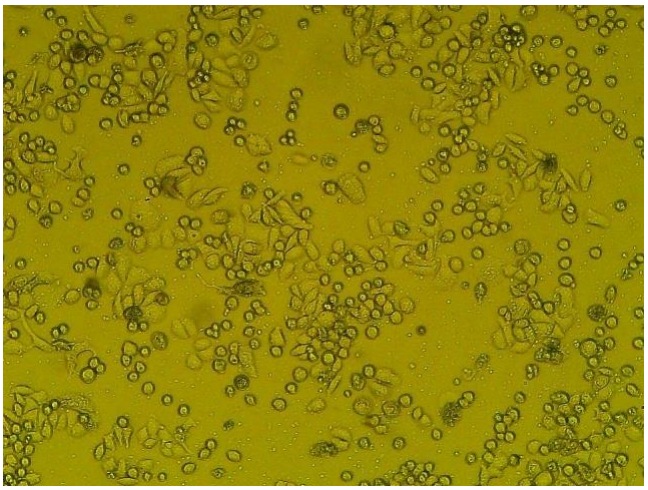
Fig 12: Zone of inhibition of different extracts on clinically isolated fungi.

Anticancer activity of biosynthesized CuONPs from aqueous extract of *T. calophylla:*

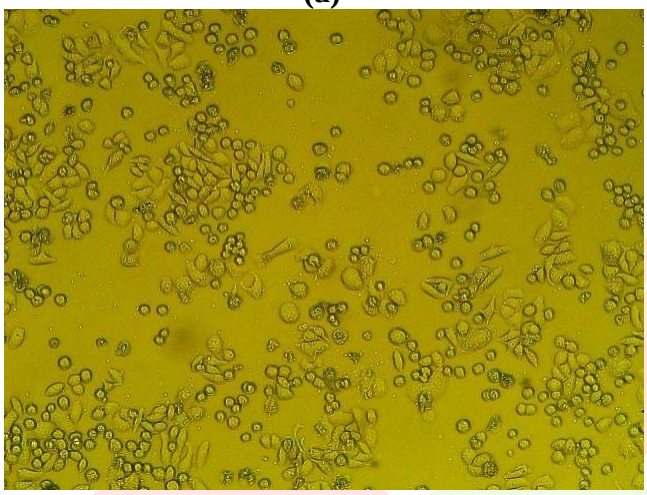
The MDA MB 453 (human cancer cell line) cell lines were used for cytotoxicity analysis by reading Formazan crystals formed by the reaction of mitochondrial dehydrogenase by MTT assay. At 48 hrs of incubation period, a significant abatement in cell viability was observed against the treated cell lines, when the concentration of CuONPs was increased from 12.5, 25, 50, 100 and 200 µg/ml. DMSO was used as a positive control to exhibit 100% of healthy proliferated cells **(Figs.13&14 , & Table4).** The 51.02 µg/ml concentration (IC_{50}) of CuONPs may 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. The Observations in Statistical data of Cytotoxicity study by ELISA Reader suggesting us that against MDA MB 453 (human cancer) cell lines, *T.calophylla* CuONPs showing good cytotoxic potential properties with the IC_{50} Concentrations at 51.02 µg/ml. But so far there is no report on CuONPs synthesized from *T. calophylla* to attribute anticancer activity against MDA MB 453 (human breast cancer) cell lines. From this study, the green synthesized CuONPs from rhizome extract showed small size spherical shaped particles, which exhibit strong cytotoxic activity against MDA MB 453 with maximum inhibition of 72.9% cell death and 27.1% cell viability respectively.



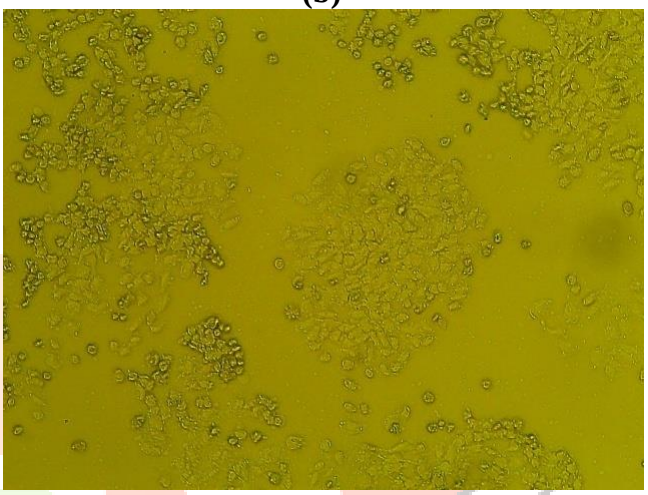
(a)



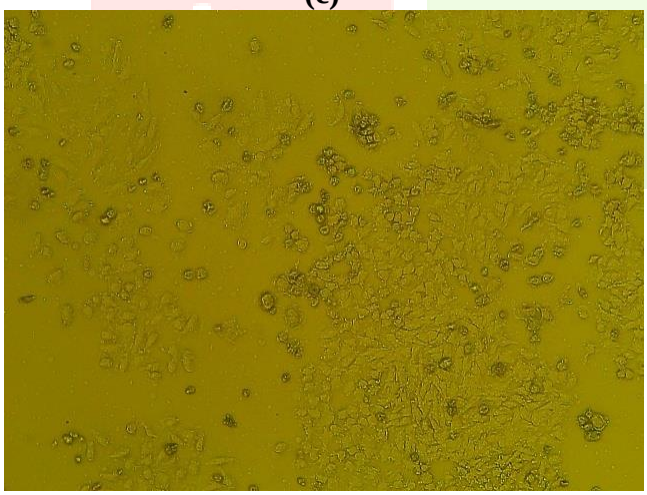
(b)



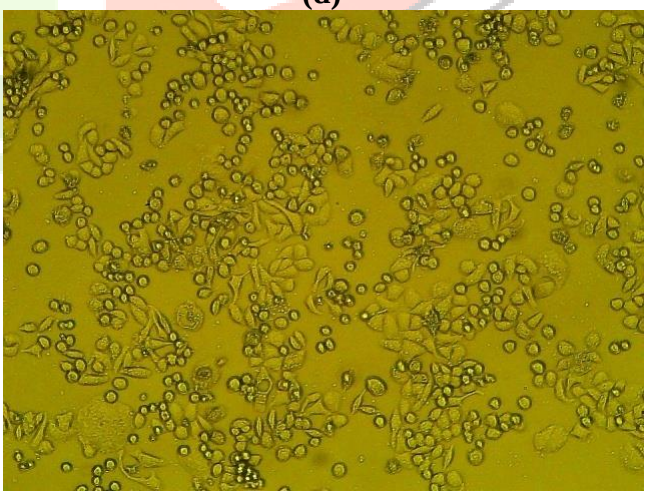
(c)



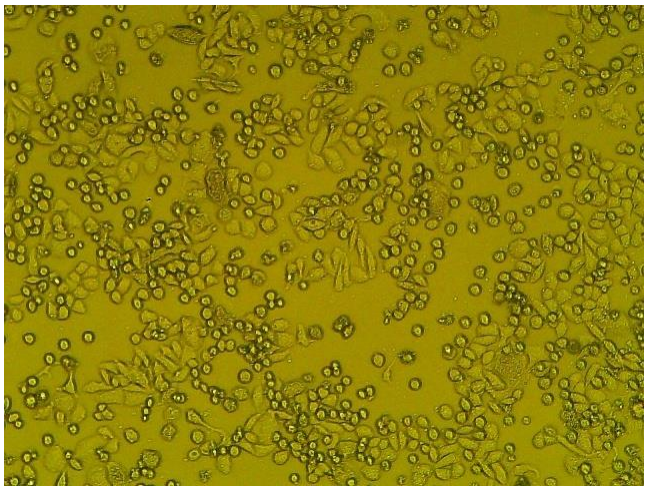
(d)



(e)



(f)



(g)

a) 12.5µl/ml

b) 25 µg /ml

c) 50 µg/ml

d) 100 µg/ml

e) 200 µg/ml

f) DMSO

g) MDA MB 453

Fig13: Anticancer activity of synthesized CuONPs from *T.calophylla*

Table 4: Anticancer effect of ***T.calophylla*** *CuONPs* on MDAMB 453 cell line (Human Breast cancer) by MTT assay.

Concerntration	Absorbence (O.D)	Cell viability (%)	Cell Death (%)
DMSO	0.833	100	**0**
12.5	0.6875	82.53	**17.47**
25	0.5625	67.52	**32.48**
50	0.425	51.02	**48.98**
100	0.2805	33.67	**66.33**
200	0.1425	27.1	**72.9**
Camptothecin	0.41	23.18	**76.82**

Graphical representation of anticancer activity from *T.calophylla*- CuONPs

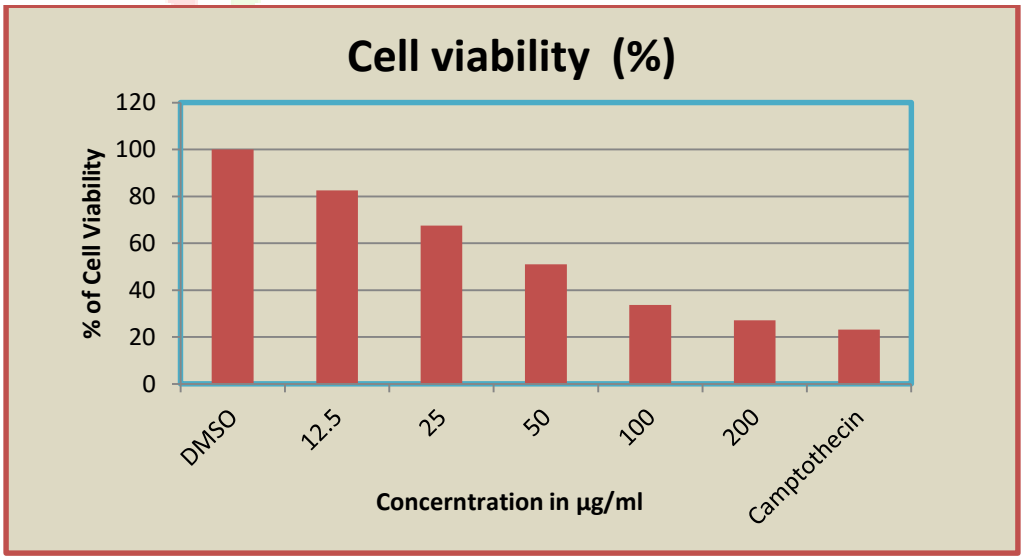


Fig 14: Anticancer activity of green synthesized CuONPs from rhizome extract of ***T.calophylla***

DISCUSSION

T. Calophylla is having hepatoprotective activity [19]. *T. calophylla* root chloroform extracts showed antibacterial and antifungal activity, at 200 mg/ml may be due to the presence of isoflavones like caloisoflavones [22]. The cytotoxicity on RAW & HT-29 cell lines by *T. calophylla*, showed significant activity [38]. Leaf extract of *T. purpurea* synthesized CuONPs (Copper oxide nanoparticles) showed effective antimicrobial activity against *Pseudomonas spp.* and *Penicillium spp.* [39]. Different flavonoids isolated from *T. calophylla* are responsible for antiprotozoal activity on *Trypanosoma*, *Leishmania* and *Plasmodium* parasites [18]. *T. calophylla* contains a wide variety of flavonoids and isoflavonoids. According to Ayurveda, this plant exhibits several medicinal properties such as antihelmintic, antipyretic, antiulcer, antimicrobial, anticancer and hepatoprotective activity. It is also active against leprosy, ulcers, and used as alternative to cure the diseases of the liver, spleen, heart and blood. The roots having diuretic, enriches blood, cures diarrhea and is useful in bronchitis, inflammations, antidiabetic, boils and pimples. Leaves are tonic to intestines and a promising appetizer [16].

CONCLUSION AND RECOMMENDATIONS

In this investigation we report a green chemistry approach for the synthesis of CuO NPs using *T. calophylla* rhizome extract. This is simple, reliable, clean method which promotes green industrial level product of CuONPs and environmentally friendly route for synthesis of benign nanoparticles. Peaks in XRD profile and bright spot array in the SAED pattern evidenced the crystalline nature of the CuONPs. The presence of flavonoids and proteins are possibly the key factors for the formation of CuO NPs. FTIR spectrum confirms the biomolecules are responsible for reducing and capping of CuONPs. From TEM measurements, the size of CuO NPs was found to be 5.97-9.32 nm is in good agreement with XRD results. Moreover, synthesized CuO NPs were pure and showed good antimicrobial and anticancer activity leads to go high potential uses in biological applications. *T. calophylla* having a vast amount of secondary metabolites such as Coumestan flavonoid, Quercetin and Rutin shows significant activities such as Hepato protective, antimicrobial, anti protozoal, anti cancer and anti HIV, anthelmintic, and anti ulcer activities. The synthesized nanoparticles of *T. calophylla* may be tested for the therapeutic applications of various biological activities for the better treatment with biosynthesized components.

ACKNOWLEDGEMENT

The authors are highly grateful to DST-PURSE, Sri Venkateswara University-Tirupati, Stellixir Biotech Pvt Ltd – Bangalore and VIT-Vellore for providing technical support

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