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ANTIMICROBIAL AND ANTICANCER ACTIVITY STUDIES ON GREEN SYNTHESIZED COPPER OXIDE NANOPARTICLES FROM THE MEDICINAL PLANT *CYATHEA NILGIRIENSIS* HOLTTUM

PRADHEESH G[1*], SURESH J[2], SURESH S[3] AND ALEXRAMANI V[4]

1: Department of Chemistry, SNS College of Technology, Coimbatore – 641035, Tamil Nadu, India

2: Department of Chemistry, Sri Ramakrishna Engineering College, Coimbatore – 641022, Tamil Nadu, India

3: Department of Chemistry, National College, Trichy-620002, Tamil Nadu, India

4: Department of Chemistry, St. Joseph's College, Trichy – 620002, Tamil Nadu, India

***Corresponding Author: E Mail: pradheeshchem@gmail.com; Tel.: +91-9047637329**

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ABSTRACT

The present work aims to synthesize biocompatible Copper (II) Oxide (CuO) nanoparticle using the extract of the medicinal plant *Cyathea nilgiriensis* Holttum. The synthesized Nanoparticle is characterized by FTIR, XRD, PSA, SEM and EDX studies. FT-IR studies confirm the presence of biomolecules and metal oxide in the prepared nanoparticle. X-ray diffraction (XRD) structural analysis reveals the formation of pure hexagonal phase structures of CuO nanoparticles. Particle size analyzer (PSA) studies have proved that the biosynthesized CuO nanoparticle is below 100 nm in size. The surface morphology of CuO nanoparticles observed through Scanning electron microscope (SEM) suggests that most of the CuO crystallites are flower -shaped. Energy Dispersive X-Ray (EDX) analysis confirms the presence of both copper and oxygen. The possible mechanistic schemes of copper oxide nanoparticles are also predicted. The biosynthesized copper oxide nanoparticles exhibit strong antimicrobial behavior against gram positive and gram negative bacterial species and fungal species. The anticancer activity against Daltons lymphoma ascites (DLA) cells has

been studied for the green-synthesized CuO nanoparticles. The anticancer activities exhibit good behavior against DLA cell lines.

Keywords: *Cyathea nilgiriensis* Holttum, CuO nanoparticles, green chemistry, Characterization, antimicrobial & anticancer activity

1. INTRODUCTION

An Eco-friendly biosynthesis means a method which doesn't make use of any toxic chemicals in the synthesis protocols. The synthetic methods based on naturally occurring biomaterials offer an alternative means for obtaining precisely required nano-particles. Generally, synthesis of nano-particles with controlled size and morphology is a challenging task which is highly dependent on the design of the protocols. Different feasible methodologies have been designed for the fabrication of nano-particles with unique size dependent properties. Due to the increasing environmental concerns, attempts have been routinely made to develop nanoparticle synthesis using greener synthesized nanoparticles using plant extracts over chemical synthesis routes **[1, 2]**. Metal nanoparticles have wide range of application in areas like agriculture, energy, environment and medicine **[3]**. Many methods like sol-gel, precipitation-stripping, solid state reaction, alkoxide-based synthesis, sonochemical preparation, microwave irradiation and precipitation–pyrolysis are adopted to prepare CuO nanoparticles **[4-6]**.

CuO nanoparticles are important because of their characteristics such as high-T_c super conductors **[7]**, sensors **[8, 9]** and catalysts **[10]**. The copper oxide nanoparticles are observed to behave as antimicrobial and antifungal agents **[11]**. Human beings have been using copper (Cu) and copper complexes for various purposes for centuries, such as water purifiers, algaecides, fungicides, and as antibacterial and antifouling agents. Copper-based compounds are efficient biocidal properties, which are generally used in several health related application **[12-14]**. Biosynthesis of copper oxide nanoparticles using microorganisms such as bacteria, fungi, yeast have been reported in the literature **[15-17]**. *Carcia papaya* leaf extract **[18]**, Aloe vera leaf extract **[19]**, *Centella asiatica* leaf extract **[20]**, *Malva sylvestris* leaf extract **[21]**, *Rosa sahandina* broth extract **[22]** and *Gloriosa superba* L. plant extract **[12]** has attracted few researches in the synthesis of copper oxide nanoparticles. The present feasibility study is to bring out a novel green synthetic strategy to prepare CuO nanoparticles using C*yathea nilgiriensis* plant extract focusing on the

application in industry. Based on the literature survey it is evident that the study on the bio-synthesis and characterization of CuO nanoparticles using *Cyathea nilgiriensis* Holttum extract has been carried out for the first time. Antibacterial activities of greener synthesizes Copper oxide (CuO) nanoparticles were tested against 3 gram positive (*S. aureus*, *B. subtilis* & *M. luteus*) and 3 gram negative (*E. coli*, *S. paratyphi* & *K. pneumonia*) bacteria using disc diffusion method. Similarly, the antifungal analysis has been done against 2 strains namely *C. albicans* and *A. niger*. The anticancer activity of biosynthesized Copper oxide (CuO) nano particles were also carried out by tryphan blue assay method using Daltons lymphoma ascites (DLA) bearing mice.

2. MATERIALS AND METHODS

2.1. Plant materials collection and preparation

The pure and analar - grade Copper nitrate [Cu $(NO_3)_2.9H_2O$] was used for this study. The pure and shade-dried leaves of *Cyathea nilgiriensis* (2 g) were powdered and subjected to extraction using deionized water. The extract obtained was filtered through Whatman No. 1 filter paper and stored in a refrigerator for further use.

2.2. Synthesis of CuO nanoparticles

A stock solution of 0.1 M $Cu(NO_3)_2.9H_2O$ was prepared. 50 ml of 0.1 M copper nitrate solution was taken in a beaker then 10 mL of *Cyathea nilgiriensis* aqueous extract was added drop wise into the solution under magnetic stirring at 80^oC for 4 hours. The complex formed was ultra-centrifuged at 10,000 rpm for 10 min. The complex residue was rinsed with water and centrifuged again at 5,000 rpm for 10 min. The complex residue was dried in an oven at 40 °C for 8 h and was then calcined in a muffle furnace at 450 °C to obtain biosynthesized CuO nanoparticles.

2.3. Instrumentation (PSA, FT-IR, XRD and SEM-EDX)

Nanoparticles size was confirmed by using particle size analyzer (Nanophox, Sympatec, Germany). The biosynthesized CuO nanoparticles were analyzed for IR characteristics using a Nicolet 520P FT-IR spectrometer set to be in the range of 500 – 4,000 cm^{-1}. A powder XRD analysis was carried out on a PAN analytical X-ray diffractometer operated at 40 kV with a current of 30 mA under Cu-K_α radiation of a 2θ range of 10–80°. The SEM images were recorded using a JEOL JSM 6390 system. An energy dispersive X -ray spectroscopic (JED 2300, JEOL) study was done on CuO nanoparticles to confirm the presence of the constituent elements – copper and oxygen.

2.4. Antimicrobial activity

The antimicrobial activity was studied applying the disc diffusion method **[23]**. Ciprofloxacin and fluconazole were taken as a standard for antimicrobial activity. A panel of 6 common pathogenic bacteria consisting of three gram-positive type S. *aureus* (NCIM 2079), *B. subtilis* (NCIM 2063), *M. luteus* (MCIM2169) and three gram negative type *E. coli* (NCIM 2065), *S. paratyphi* (NCIM 2501), *K. pneumoniae* (NCIM2707) and 2 fungal strains *C. albicans* (MTCC 3100) *and A.niger* (MTCC 1344) was used. These microbial strains were obtained from the Kovai Medical College and Hospital (KMCH), Coimbatore, Tamil Nadu, India.

2.5. Anticancer activity

Trypan blue dye assay method **[24, 25]** was carried out to evaluate the in vitro cytotoxicity potentials biosynthesized CuO nanoparticles. The cells were aspirated from the peritoneal cavity of tumor bearing mice. The cells were washed three times using PBS and the viability of the cells was checked using trypan blue. Different concentrations (10, 20, 50, 100 and 200µg) of CuO nanoparticles were prepared. In a test tube, 100µl of CuO nanoparticles was mixed with 800µl of phosphate buffer saline and 100µl ($1X10^6$ in 1ml) of Dalton's Lymphoma Ascites (DLA) was added. All the test tubes were incubated at 37°C in an incubator for 3 hours. About 100µl of trypan blue dye was added to all the test tubes. Dead cells took on a trypan blue color while live cells did not absorb the dye. The number of stained and unstained cells was measured using hemocytometer. Percentage of cytotoxicity was calculated by the following formula.

$$Cytotoxicity\ (\%) = \frac{N_d}{N_d + N_l} \times 100$$

Where, N_d - No of dead cells, N_l - No of live cells

3. RESULTS AND DISCUSSION

3.1. Synthesis mechanisms of copper oxide nanoparticles

Figure 1 shows the possible mechanism for the formation of copper oxide nanoparticles. Preliminary phytochemical analysis of C. *nilgiriensis* ethanol extracts confirmed the presence of Tannin, Saponnin, Flavonoids, Steroids, Terpenoids, Triterpenoids, Carbohydrate, Protein, Anthroquinone, Polyphenol, Glycoside and Coumarine. The flavonoid type of compounds could have formed complex with copper (II) ion of copper Nitrate solution. The complex solution was then heated in hot air oven for 8 hours to form copper hydroxide further it was calcinated at 450°C to form copper oxide nanoparticles.

3.2. Particle Size Analyzer

The average size of the CuO

nanoparticles and the statistical distribution of the size were determined using the particle size analyzer. The particle size of Copper oxide nanoparticles is found to be below 100 nm. The results are shown in **Figure 2**.

3.3. FT-IR spectral study

Figure 3 Shows the FT-IR spectrum of bio-synthesized copper oxide nanoparticles. Copper Oxide absorbs IR radiation at 513.09 cm^{-1}. The O-H stretch appears in the spectrum at 3449.84 cm^{-1}. The absorption in the region 1632.81 cm^{-1} indicates the presence of aromatic ring. The absorption peak at 1113.94 cm1 corresponds to C-O stretching of saturated primary alcohol. The band 1632.81 cm-1 corresponds to the carbonyl group of flavonoids. The results of FTIR analysis indicated that phenolic type of compound would have a stronger binding force towards the metal, favoring the formation of metal nanoparticles and minimizing the agglomeration. This suggests that the phenolic type of biological molecules could do dual functions of formation and stabilization of copper oxide nanoparticles in aqueous medium.

3.4. X-ray diffractometer

Figure 4 shows the X-ray diffractometer (XRD) spectrum of the biosynthesized copper oxide nanoparticles. The main peaks found correspond to Bragg reflections with 2θ values of 32.49°, 35.52° 38.71°, 46.30°, 48.80°, 53.36°, 58.20°, 61.52°, 66.21°, 67.87°, 72.30°, 75.05°,80.46,82.52° and 86.95°. Locations of the characteristic Bragg reflections were indexed to (1 1 0), (-111), (1 1 1), (-1 0 0), (-2 0 2), (0 2 0), (2 0 2), (-1 1 3), (-31 1), (2 2 0), (311), (-2 2 2), (-3 1 3), (2 2 2) and (1 3 0) planes of CuO monoclinic phase structures, respectively (standard JCPDS card 01-080-1916) and this confirms the presence of copper oxide nanoparticles. The XRD pattern observed confirms the absence of other impurities. **Table 1** shows the calculated crystallite sizes and lattice strain values for CuO nanoparticles found based on Debye Scherer's formula, which is given below.

$$D = 0.94\lambda/\beta Cos\theta$$

Where D is the average crystallite size, where ß denotes line broadening in radians, where θ is the Bragg angle, and where λ is the X-ray wavelength. The average crystallite size observed from the intense plane of (-1 1 1) was measured as 29.05 nm. XRD patterns obtained through this study are similar to XRD patterns obtained for biologically synthesized CuO nanoparticles **[26]**.

3.5. SEM and EDX analyses

Raja Naika and colleagues found a spherical shaped nanoparticle with a diameter range of 5-10 nm in Gloriosa

superba L. plant extract **[27]**. **Figure 5 a, b, c & d** shows SEM and EDX images of biosynthesized copper oxide nanoparticles. The SEM images show agglomerations of individual copper oxide particles. A closer look at the agglomerated lump shows the presence of several nanoparticle aggregates. In **Figure 5 (a)**, particles appear to be agglomerated and some individual crystals are clearly visible. In **Figure 5 (b) & (c)** some particles appear to be in flower shaped. The EDX results shown in **Figure 5(d)** further confirm the presence of both copper and oxygen in copper oxide nanoparticles without any impurities.

3.6. Antimicrobial activity of CuO nanoparticles

H. Raja Naika *et al*. **[12]** found significant antibacterial activity in CuO nanoparticles synthesized from Gloriosa superba L extract against pathogenic bacterial strains Gram negative (*Klebsiella aerogenes*, *Pseudomonas desmolyticum*, and *Escherichia coli*) and Gram positive bacteria (*Staphylococcus aureus*). Similarly, CuO nanoparticles synthesized *Ocimum basilicum* plant leaf extracts exhibited good antibacterial behavior against pathogenic bacterial strains *E. coli* and *S. aureus* **[27]**. Faheem Ijaz *et al*. **[28]** found good antibacterial activity for CuO nanoparticles synthesized from Abutilon indicum against the bacterial species such as *Klebsiella and Bacillus subtilis.* In this study we explored antimicrobial activity of CuO nanoparticles synthesized using medicinal plant Cyathea nilgiriensis leaf extract. CuO nanoparticles exhibit antimicrobial efficiency due to the factors such as particle size and surface morphology.

We also found biosynthesized CuO nanoparticles obtained from Cyathea nilgiriensis plant extract to exhibit good antibacterial behavior. **Figure 6** shows the antibacterial activity of the biosynthesized CuO nanoparticles. Antibacterial activity was measured against three grams of positive bacteria (*S. aureus, B. subtilis, M. luteus*) and three gram of negative bacteria (*E. coli, S. paratyphi, K. pneumoniae*). Antifungal activity was carried out against *C. albicans* and *A. niger*, Ciprofloxacin is taken as the standard for all microbial strains.

Table 2 shows inhibition results obtained for the CuO nanoparticles. The zone of inhibition for standard ciprofloxacin for three gram positive bacteria was found to be 34 mm (*S. aureus*), 27 mm (*B. subtilis*) and 36 mm (*M. luteus).* The zones of inhibition of CuO nanoparticles for three gram positive bacteria are found to be 20 mm (S. aureus), 17 mm (B. subtilis) and 21 mm M. luteus. When we compare the zone of inhibition of three gram positive bacteria, *M. luteus (21 mm)* shows a larger

zone of inhibition. The zone of inhibition for standard ciprofloxacin for three gram negative bacteria ranges from 36 mm (*E coli)*, 35 mm (*S. paratyphi)* and 32 mm (*K. pneumoniae*). The zones of inhibition CuO nanoparticles for three gram negative bacteria are found to be 20 mm (E coli), 16 mm (S. paratyphi) and 19 mm (K. pneumoniae). On comparing the zone inhibition values for three gram negative bacterial species E. *coli* (20 mm) shows a larger zone of inhibition. This superior activity against bacterial species was due to the fact that the copper ions released form CuO nanoparticles permeated the bacterial cell membrane and destroyed the structure of the cell membrane by attaching to the negatively charged cell wall **[29, 18]**. Copper ions are involved in cross-linkage of nucleic acid strands by binding them with DNA molecule of bacteria. This results in a disordered helical structure of DNA molecule which causes denaturation of proteins and some other biochemical processes in the cell, leading to complete destruction of the bacterial cell **[30]**.

Table 3 shows antifungal results for the biosynthesized CuO nanoparticles. **Figure 7** shows antifungal images of the CuO nanoparticles. The zone of inhibition for the standard Ciprofloxacin of two fungal species are found to be *C. albicans* (35 mm) and *A. niger* (09 mm). *C.albicans* (24 mm) presents a larger zone of inhibition compared to that of *A. niger* (10 mm) for the biosynthesized CuO nanoparticles. Antifungal activity is likely derived through electrostatic attraction between the negatively charged cell membranes of microorganisms and the positively charged nanoparticles **[31, 32]**. The biosynthesized copper oxide nanoparticle synthesized using *Cyathea nilgiriensis* Holttum leaf extracts has effective inhibition against the fungal species. Thus, nanoparticles can be used as antifungal agents and can help to overcome hurdles of fungal diseases in human beings.

3.7. Anticancer activity

The potential anticancer activity of silver nanoparticles synthesized using different plant extracts had been reported in literature **[33, 34]**. In this work the focus was on CuO nanoparticles synthesis using *Cyathea nilgiriensis* Holttum leaf extract and their potential anticancer behavior against the mice cell lines. **Figure 8 [a-f]** shows anticancer images of CuO nanoparticles at different concentrations. DLA (Daltons lymphoma ascites) bearing mice cell lines was used for determining cancer activity based on different concentrations (10, 20, 50, 100 and 200µg) of CuO nanoparticles. The cyclophos-phamide drug is taken as a standard for the DLA cell line. **Figure 8a** shows a control

image and there is no inhibition here. However, in **Figure 8 [b-f]** clearly shows that CuO nanoparticles have increasing percentage of inhibition over higher concentration of the test sample. At 200µg/ml we observed the maximum inhibition rate for CuO nanoparticles. In this work we found biosynthesized CuO nanoparticles exhibit strong anticancer activity. **Table 4** shows the anticancer activity results of CuO nanoparticles.

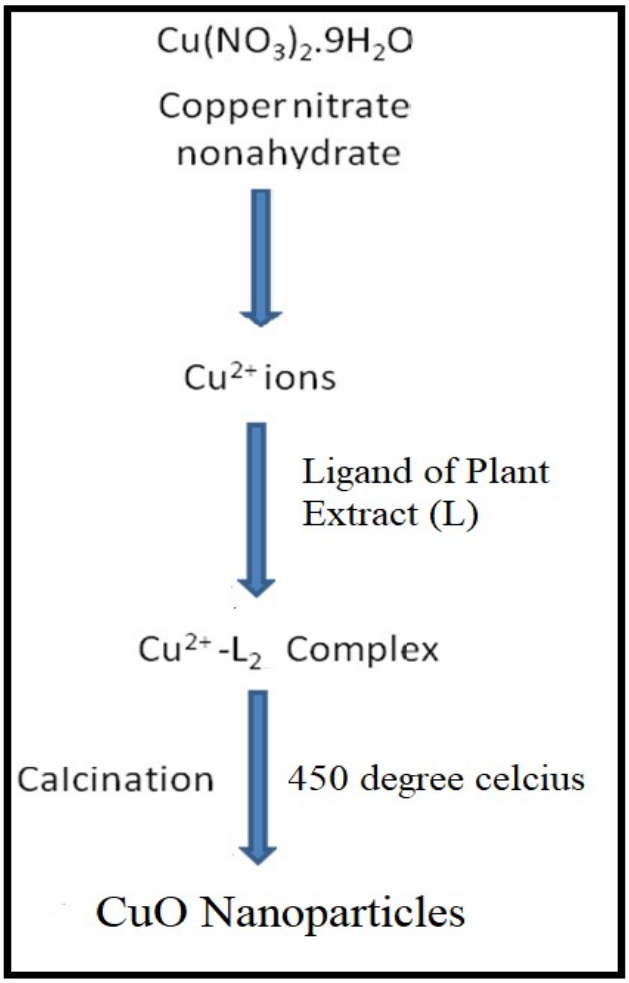

Figure 1: Scheme of CuO Nanoparticle synthesis

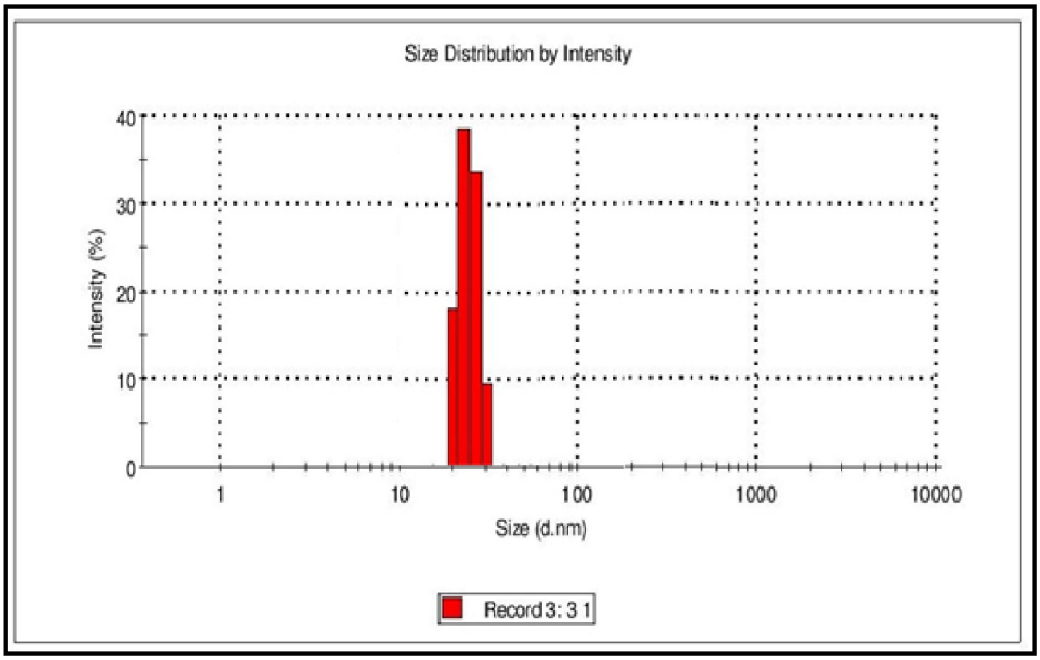

Figure 2: PSA image of CuO nanoparticles

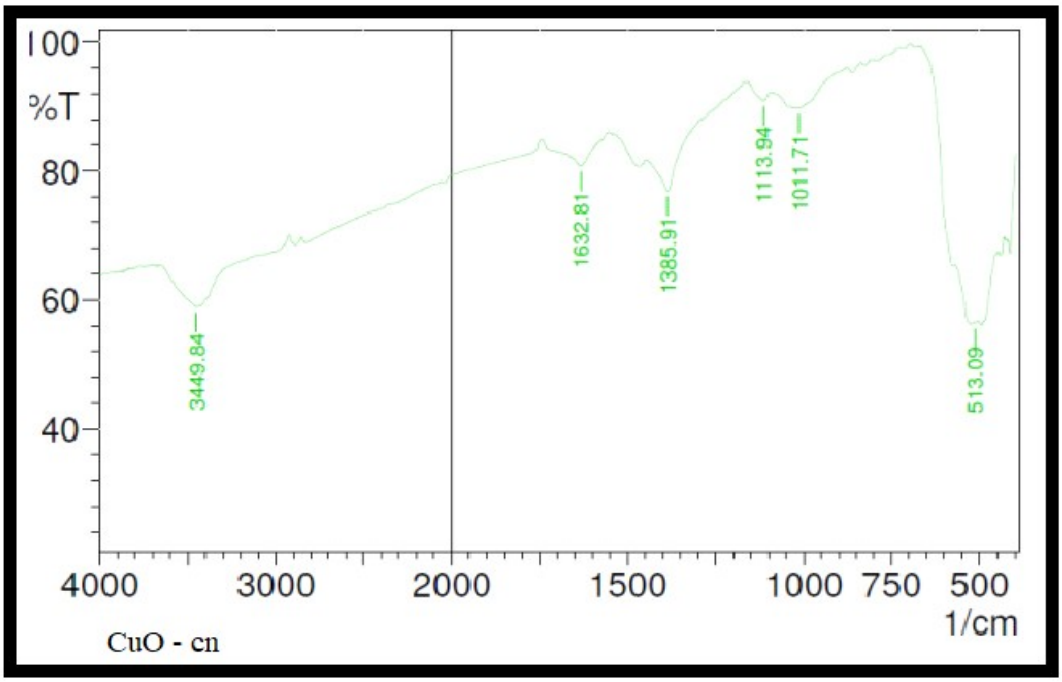

Figure 3: FT-IR spectra of CuO nanoparticle

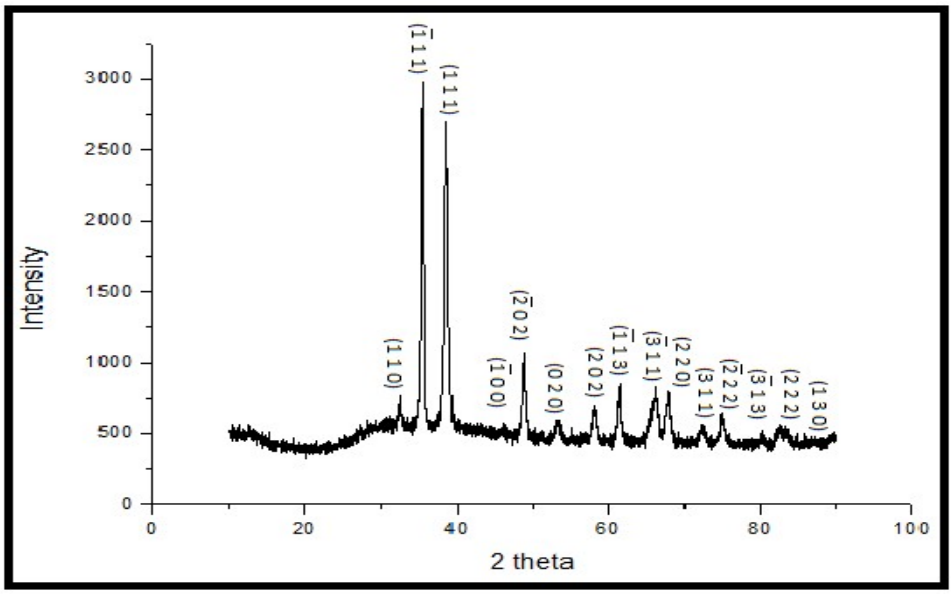

Figure 4: XRD spectra of CuO nanoparticle

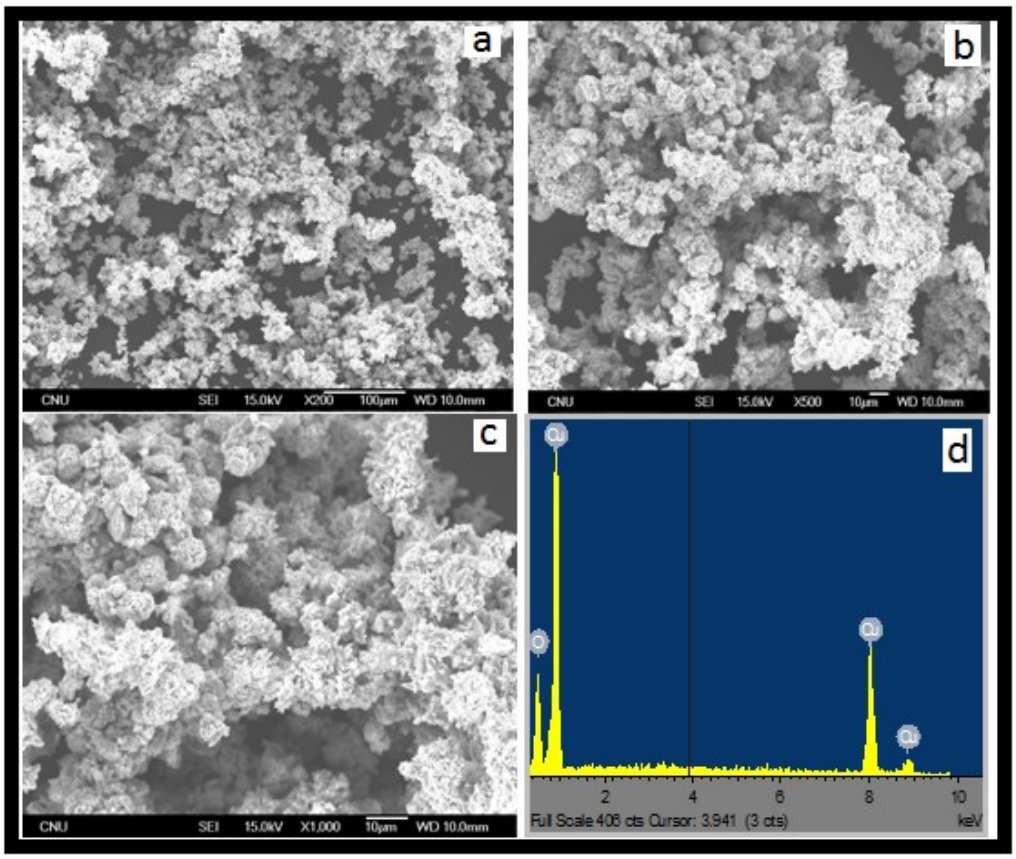

Figure 5: SEM (a, b & c) & EDX (d) spectra of CuO nanoparticles

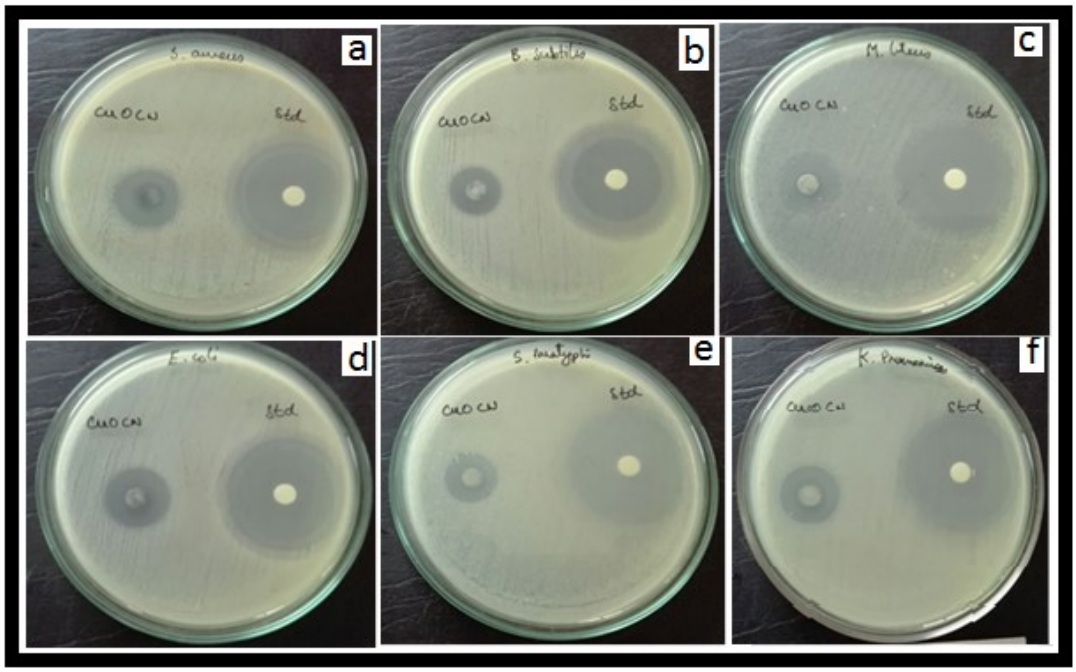

Figure 6: Antibacterial images of CuO nanoparticles a) *S. aureus*, b*) B.subtilis*, c) *M.luteus* d) *E. coli* e) *S.paratyphi* f) *K.pneumoniae*

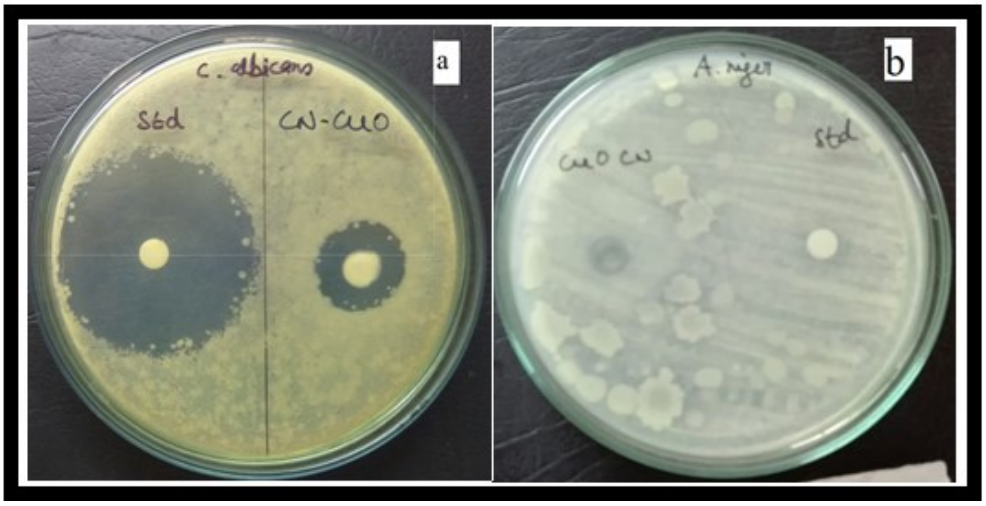

Figure 7: Antifungal images of CuO nanoparticles a) *C.albicans* b) *A.niger*

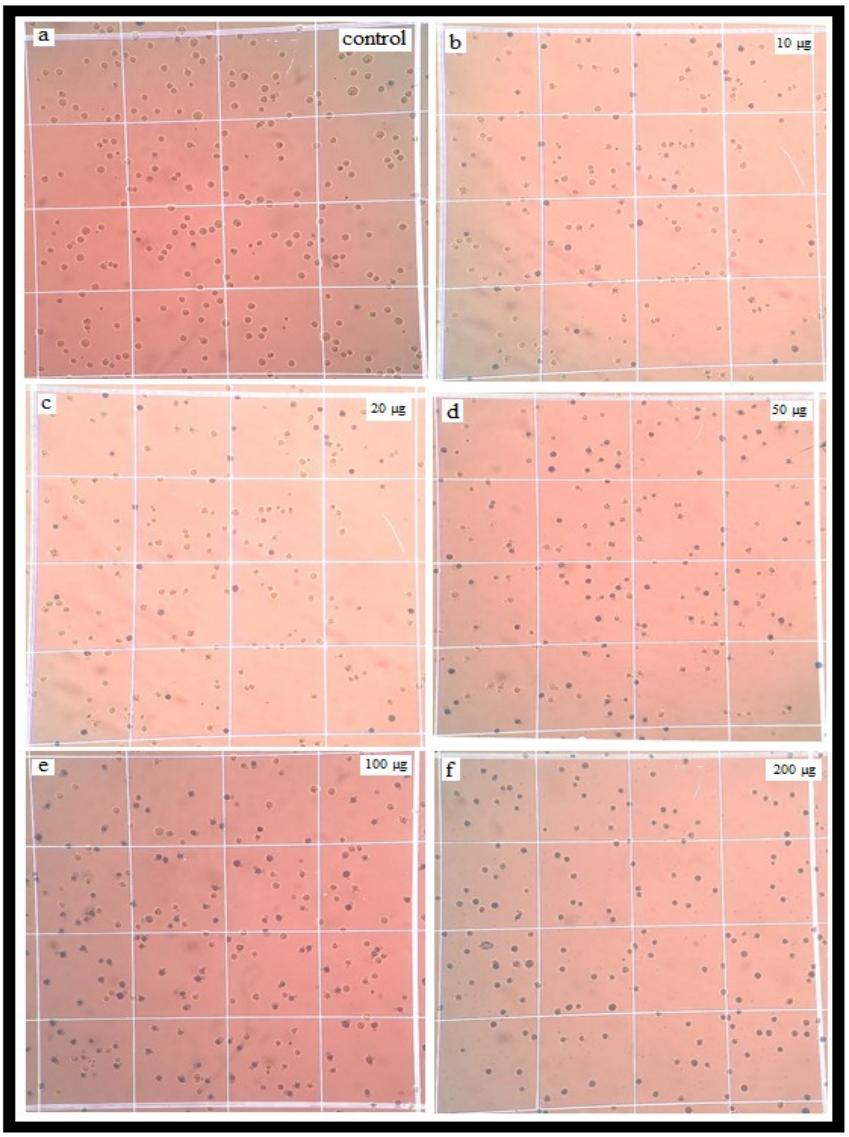

Figure 8: Anticancer activity of CuO nanoparticles a) Control b) 10µg c) 20 µg d) 50 µg e) 100 µg f) 200 µg

Table 1: XRD Values of CuO nanoparticles

2 theta	Crystallite size (nm)	Lattice strain
32.49	28.82 (110)	0.0045
35.52	29.05 (-111)	0.0041
38.71	29.33 (111)	0.0037
46.30	30.08 (-100)	0.0031
48.80	30.38 (-202)	0.0029
53.36	30.96 (020)	0.0026
58.20	31.66 (202)	0.0024
61.52	32.20 (-113)	0.0022
66.21	33.03 (-311)	0.0020
67.87	33.35 (220)	0.0019
72.30	34.26 (311)	0.0018
75.05	34.88 (-222)	0.0017
80.46	36.23 (-313)	0.0015
82.52	36.80(222)	0.0015
86.95	38.12 (130)	0.0014

Table 2: Zone inhibition values of gram positive and gram negative bacterias

Name of organisms	Zone of Inhibition (mm)	
	Std Ciprofloxacin (10µg/disc)	Samples (100µg/disc)
		CN - CuO
S. aureus	34	20
B. subtilis	27	17
M. luteus	36	21
E. coli	36	20
S. paratyphi	35	16
K.pneumoniae	32	19

Table 3: Zone inhibition values of fungal species

Name of organisms	Zone of Inhibition (mm)	
	Std Ciprofloxacin (10µg/disc)	Samples (100µg/disc)
		CN - CuO
A.niger	09	10
C.albicans	35	24

Table 4: Anticancer activity results of CuO nanoparticles

Cell line	Name of sample		Anticancer Results					
DLA	CuO nanoparticles	Concentration µg /ml	Control	10	20	50	100	200
		% of inhibition	100	20	35	70	82	100

4. CONCLUSIONS

The biocompatible synthesis of copper oxide nanoparticles (CuO) was achieved by applying a simple and novel green chemistry procedure involving the use of *Cyathea nilgiriensis* Holttum leaf extract as a reducing and capping agent. The biosynthesis of CuO nanoparticles was carried out via hydroxide precipitation at room temperature followed by calcination at 450°C. The successful formation of copper oxide nanoparticles was confirmed by PSA, FT-IR, XRD, SEM and EDX analyses. XRD results confirms the average crystallite size observed from the intense plane (101) was measured as 29.11 nm. The biosynthesized CuO nanoparticles exhibited strong levels of antibacterial activity against M. *luteus* (Gram-positive bacteria) and E. *coli* (Gram-negative bacteria). *C.albicans* exhibited stronger antifungal behavior than the C. *albicans* strain. Biosynthesized CuO nanoparticles were found to protect against bacterial and fungal pathogens, suggesting that they may be used as effective antimicrobial and anticancer agents for commercial biomedical applications.

5. ACKNOWLEDGMENT

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6. CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

[1] Iravani S. Green Synthesis of Metal Nanoparticles Using Plants, Green Chemistry, 13, 2014, 2638-2650.

[2] Susan A, Mansor B A, Farideh N, R osfarizan M, Biosynthesis of Silver Nanoparticles Using Brown Marin e *Macroalga, Sargassum Muticum* Aqueous Extract, Material letter, 6, 2013, 5942-5950.

[3] Chandran S P, Chaudhary M, Pasricha R, Ahamed A, Sastry M, Synthesis of Gold Nanotriangles and Silver Nanoparticles Using Aloe vera Plant Extract, Biotechnology Progress, 22, 2006, 577-583.

[4] Pike J, Chan SW, Zhang F, Wang X, Hanson J, Formation of stable Cu_2O from reduction of CuO nanoparticles, Applied Catalysis A, 303, 2006, 273-277.

[5] Zhou K, Wang R, Xu B, Li Y, Synth esis, characterization and catalytic properties of CuO nanocrystals w i t h v a r i o u s s h a p e s , Nanotechnology, 17, 2006, 3939-3943.

[6] Wu Q H, Wei X W, Shao M W, Gu J S, Qu M Z, Preparation of Fe–Ni alloy nanoparticles inside carbon nanotubes *via* wet chemistry, Chem Phys Lett, 12, 2002, 1919-1921.

[7] Yip S K, Sauls J A, Nonlinear Meissner effect in CuO superconductors, Physical Review Letter, 69(15),1992, 2264-2267

[8] Kim Y S, Hwang I S, Kim S J, lee C Y, Lee J H, CuO nanowire gas sensors for air quality control in automotive cabin, Sensors and Actuators B: Chemical, 135, 2008, 298-303.

[9] Umar A, Rahman M M, Hajry A Al, Hahn Y B, Enzymatic glucose biosensor based on flower-shaped copper oxide nanostructures composed of thin Nano sheets, Electrochemistry. Communications, 11, 2009, 278-281.

[10] Yang S, Wang C, Chen L, Chen S, Facile dicyandiamide-mediated fabrication ofwell-defined CuO hollow microspheres and their c atalytic application, Materials C hemistry and Physics,120,2010,2 96-301.

[11] Borkow G, Zatcoff R C, Gavia J, Putting copper into action: copp er-impregnate-d products with p o t e n t b i o c i d a l a c t i v i t i e s , Medical Hypotheses, 9,2009, 4.

[12] Raja Naika H, Green synthesis of CuO nanoparticles using *Glorios a superba* extract and their anti-b acterial activity, Journal of Taiba h University for Science, 9, 2015, 7-12.

[13] Borkow G, Zatcoff R C, Gabbay J, Reducing the risk of skin pathologies in diabetics by using copper impregnated socks, Medical Hypotheses, 73,2009, 883-886.

[14] Vellora V, Padil T, Cerník M, Green synthesis of copper oxide nanoparticles using gum karaya as a bio template and their antibacterial application, International Journal of Nanomedicine, 8,2013, 889-898.

[15] Singh A V, Patil R, Anand A, Milani P, Gade W N, Biological synthesis of copper oxide nanoparticles using *E.coli*, Current Nanoscience, 6, 2010, 365-369.

[16] Rahman A, Ismail A, Jumbianti D, Magdalena, Sudrajat H, Synthesis of Copper oxide nano particles by using *Phormidium Cyanobacterium*, Indonesian Journal of Chemistry, 9, 2009, 355 - 360.

[17] Honary S, Barabadi H, Fathabad E G, Naghibi F, Green synthesis of copper oxide nanoparticles using *Penicillium aurantiogriseum*, *Pen icillium citrinum* and *cenicillium*. Digest Journal of Nanomaterials and Biostructures, 7, 2012, 999-1005.

[18] Sankar R. *et al*, Green synthesis of colloidal copper oxide nanoparticles using *Carica papaya* and its application in photocatalytic dye degradation, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 121,2014, 746-750.

[19] Gunalan S, Sivaraj R, Venckatesh R, *Aloe barbadensis* Miller mediated green synthesis of Mono disperse copper oxide nanoparticles: optical properties, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 97, 2012, 1140-1144.

[20] Devi H S, Singh T D, Synthesis of Copper Oxide Nanoparticles by a Novel Method and its Applicat ion in the Degradation of Methyl Orange, Advance in Electronic a nd Electric Engineering, 2014. 3-88.

[21] Awwad A M, Albiss B A, Salem N M, Antibacterial Activity of synthesized Copper Oxide Nanopa rticles using *Malva sylvestris* Leaf

Extract, SMU Medical Journal, 2, 2015, 91-100.

[22] Jafarirad S, Mehrabi M, Pur E R, Biological Synthesis of Zinc Oxi de and Copper Oxide Nanopartic les. International Conference on Chemistry, Biomedical and Envir onment Engineering, 2014, 62-64.

[23] Bauer A W, Kirby W M, Scherris J C, Truck, M, Antibiotic susceptibility testing by a standardized single disc method, The American Journal of Clinical Pathology, 36,1996, 493-496.

[24] Gupta, S.K. Handbook of practical and Clinical Immunology, 3rd Ed.; CBS Publishers & Distributors: New Delhi, India, 2002, 299-300.

[25] Rajkapoor B, Jayakar B, Muruges h N. Antitumour activity of *Bauh inia variegata* on Dalton's Ascitic lymphoma Journal of Ethn opharmacology, 89, 2003, 107-109.

[26] Vijay Kumar P P N, Shameem U, Pratap K, Kalyani R L, Pammi S V N, Green Synthesis of Copper O xide Nanoparticles Using Aloe vera Leaf Extract and Its antiba cterial activity against fish bacte rial pathogens, Bionanoscience, 5, 2015, 135-139.

[27] Altikatoglu M, Attar A, Erci F, Cristache C M, Isildak I, Green synthesis of copper oxide nanoparticles using *Ocimum basilicum* extract and their antibacterial activity, Fresenius Environmental Bulletin, 26,2017, 7832-7837.

[28] Ijaz F, Shahid S, Khan S A, Ahmad W, Zaman S, Green synthesis of copper oxide nanoparticles using *Abutilon indicum* leaf extract Antimicrobial, antioxidant and photocatalytic dye degradation activities, Tropical Journal of Pharmaceutical Research,16, 2017, 743-753.

[29] Ren G *et al*, Characterisation of copper oxide nanoparticles for antimicrobial applications, International Journal of Antimicrobial Agents, 33, 2009, 587-590.

[30] Yallappa S *et al*, Microwave assisted rapid synthesis and biological evaluation of stable copper nanoparticles using *T. arjuna* bark extract, Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy, 110, 2013, 108-115.

[31] Hamouda T *et al*, A novel surfactant Nano emulsion with a unique non-irritant topical antimicrobial activity against

bacteria, enveloped viruses and fungi, Microbiological Research, 156, 2001, 1-7.

[32] Dibrov P, Dzioba J, Gosink KK, Hase C C, Chemiosmotic mechanism of antimicrobial activity of $Ag^{(+)}$ in Vibrio cholera, Antimicrobial Agents and Chemotherapy, 46(8), 2002, 2668-2670.

[33] Prasannaraj G, Venkatachalam P, Green engineering of biomolecule-coated metallic silver nanoparticles and their potential cytotoxic activity against cancer cell lines, Advances in Natural Sciences: Nanoscience and Nanotechnology, 8, 2017, 025001.

[34] Ebraminezhad A, Bagheri M, Taghizadeh SM, Berenjian A, Ghasemi Y, Biomimetic synthesis of silver nanoparticles using micro algal secretory carbohydrates as a novel anticancer and antimicrobial, Advances in Natural Sciences: Nanoscience and Nanotechnology, 7, 2016, 015018.