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Green Synthesis of Copper Nanoparticles Mediated By Dioscorea Bulbifera Tuber for Biofilm Inhibition

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ABSTRACT

Herein, we report the synthesis of Cu NPs via green approach using Dioscorea bulbifera plant tuber extract. The as-synthesized products were characterized using X-ray diffraction (XRD) analysis reveals that confirmation of the face centered cubic (FCC) crystal structure without any impurity peaks with a lattice constant 0.3620 nm. Fourier Transform Infrared Spectroscopy (FTIR) shows the components like flavonoid and phenol were observed, which have contributed as surface capping agents for NPs. UV-visible spectroscopy shows that Cu NPs shows a strong surface Plasmon resonance absorption peak at 570 nm, which attributed to the formation of Cu NPs. Field emission scanning electron microscope (FE-SEM) exhibit a spherical shape of Cu NPs agglomerate morphology and Energy Dispersive X-ray analysis (EDAX) which confirm the presence of only Cu element without any impurities. Synthesized sample explored of Candida albicans biofilm and it shows about 20 % inhibition of biofilms.

Key words: Green synthesis, copper nanoparticles, Dioscorea bulbifera, biofilm and Candida albicans

I. INTRODUCTION

Nanomaterial's have unique properties that distinguish them from the corresponding bulk materials. The shape and size of metal nanoparticles influence their optical, catalytic and conductive properties [1]. Synthesis of nanoparticles through a green approach have been widely used because of its nontoxic, environment friendly and cost effective nature as compared with chemical as well as physical methods [2]. Copper nanoparticles (Cu-NPs) have applications such as super strong materials, antibacterial, sensors, catalysts, DNA binding, antimicrobial activity, antioxidants, industries and medicine [3]. There are several reports on plant mediated green synthesis of Cu NPs such as Eclipta prostrata leaves [4], Celastrus paniculatus Willd [5], Ageratum houstonianum Mill leaf [6], Cissus vitiginea [7], Juglans regia leaf [8], Lantana camara flower [9], Jatropha curcas leaves [10], Punica granatum [11] and Tea Leaf [12].

Dioscorea bulbifera is one of the unique medicinal plants among 600 species in the family Dioscoreaceae which has found its importance in traditional

medicine worldwide [13]. Among various medicinal plants used in Ayurveda, Indian system of traditional medicine as well as its multiple therapeutic potential [14]. Dioscorea bulbiferais used as alternative medicine in the various regions of India as well as Zimbabwe. Dioscorea bulbiferaplant tuber used as herbal tonic which further stimulates the stomach and spleen and also it shows effect on the lungs and kidneys. Tubers are also useful for to cure various activity such as dry cough, diabetes, asthma uncontrollable urination, poor appetite, chronic diarrhea, ulcers, anticancer, antioxidant, analgesic, and antinflammatory properties [15].

Biofilms are the bacterial groups firmly lodged in the extracellular matrices of polysaccharides, proteins, enzymes, and nucleic acids; thereby, facilitating anchorage to any surfaces irreversibly [16]. Candida albicans is considered as a major fungal pathogen of the humans causing considerable morbidity and mortality. Currently prescribed antifungal, antibiotics areoften failures in clinical situations, due to the development of multiple drug resistance, formation of drug resistant biofilms on biotic as well as abiotic surfaces and transformation from yeast to hyphal form morphology [17].

Antibiofilm Assay:

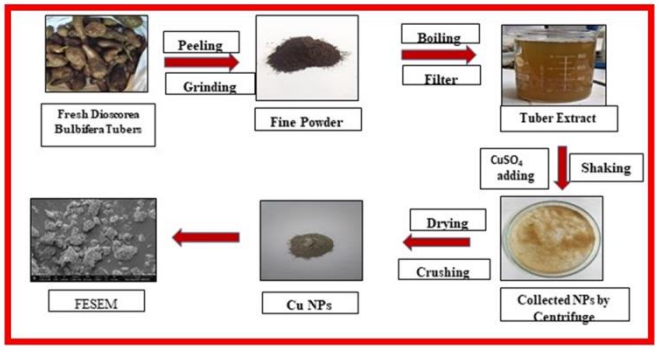
The procedure is taken from [18], typically 100µL of 107 cells/mL of Candida albicans in water were added into 96 well polystyrene plates and incubated for 3 hours for adherence of cells. The un-adhered cells were gently washed with phosphate buffer saline (PBS). 100 µL of Roswell Park Memorial Institute (RPMI) medium containing different concentrations (0–100 µg/L) of Cu NPs were added to each well, and incubated for 24 hours at37 oC. After 24 hours of incubations, the medium supernatant was removed, and the biofilm formed was gently washed with PBS and further incubated with100 µL of 5 mg/mL MTT (3-(4,5 Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) solution. After few hours of incubations, 100 µL of dimethyl sulfoxide was added into each well and optical density was recorded.

II. MATERIALS AND METHODS:

Plant material and preparation of extract Dioscorea bulbifera tubers were collected from natural geographical landscape of Dindori, Nashik region Western Ghats of Maharashtra, India and were identified and authenticated by botanist from national research institute of basic Ayurvedic science, central council for research in Ayurveda and siddha, department of ayush, ministry of Health and family welfare, Government of India, New Delhi, Nehru garden, kothrud, pune, India assigning voucher specimen number 860 [19]. Washed tubers were sliced into small pieces and shade dried for 5-7 days followed by blending into fine powder. 10 g of finely ground tuber powder was boiled with 100 ml of sterile distilled water for 5 min in a 300 ml Erlenmeyer flask. The extract obtained was filtered through whatman filter paper No. 1 the filtered sample was collected and stored at 40C

Synthesis method –

Synthesis of Cu NPs was done with slight modification 10 ml of DBTE was added to 95 ml of 1.5 Mm aqueous CuSO4.5H2O solution and kept under shaking condition at 200 rpm and reduction of Cu2+ion.



Schematics of synthesis of Cu NPs by green synthesis approach

III. RESULTS AND DISCUSSION:

1] XRD Studies

The X- ray diffraction XRD pattern of Cu NPs is shown in figure: (1). The diffraction intensities were recorded from 10 to 80o at 2θ angle, three characteristic peaks were observed at 2θ angles of 43°,50° and 74°, and these corresponds to the crystal planes (111), (200) and (220) which well matches with [Joint Committee on Powder Diffraction (JCPDS) 04-0784] The Cu NPs shows Face Center Cubic crystal structure [10].

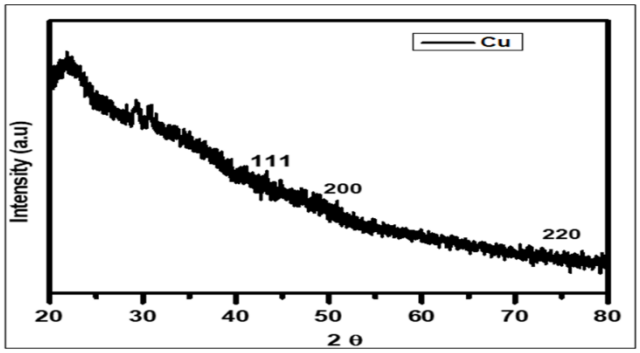


Fig 1 – XRD pattern of synthesized Cu NP

2] UV-Visible- Diffuse Reflectance (UV- Vis-DRS) Spectroscopy

From figure 2 shows the spectrum of Dioscorea bulbifera tuber powder and synthesized Cu NPs. The red spectrum indicates Dioscorea bulbifera tuber and synthesized Cu NPs shown black color. UV-Vis-DRS confirms the appearance of Cu NPs with strong surface plasmon resonance (SPR) peak at 570nm. The absorption band for Cu NPs has been reported to be in the range of 500-600 nm [20].

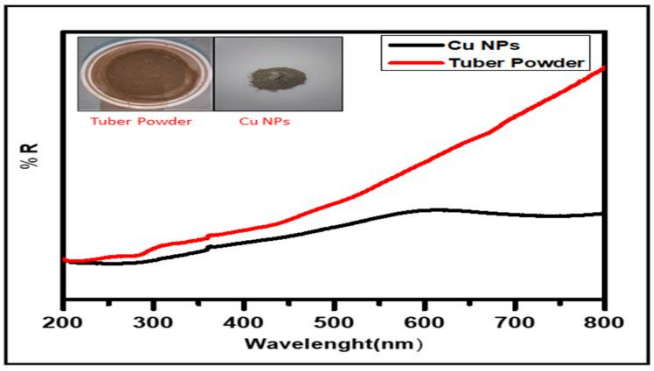


Fig 2 – DRS spectrum of synthesizes Cu NPs

3] Fourier transform infrared (FTIR) analysis:

FTIR technique is used to investigate the surface functional group of DBT powder (Dioscorea bulbifera tuber) as well as synthesized Cu NPs. FTIR spectra of DBT was recorded before synthesis and the peak shows the key components such as flavonoid and phenolic were observed in figure 3 (a): The peaks observed at 1639, 1421, 1155, 1082, and 1026 cm-1 represents C=C stretch, O-H stretch, C-O stretch and C-N stretch the broad and strong peak was found in DBT at 3495 cm-1, which is due to hydroxyl (-OH) group of phenols/alcohol.FTIR analysis also performed to recognize the various functional group present in Cu NPs mediated by Dioscorea bulbifera as shown in the figure 3(b): It also shows different stretching vibration of bands at different peaks; 3296 cm-1 O-H stretch; 2926 cm-1 C-H strong stretch vibration; 1622 cm-1 C=C variable stretch vibration; 1413 cm-1 –C-H variable bending vibration [14].

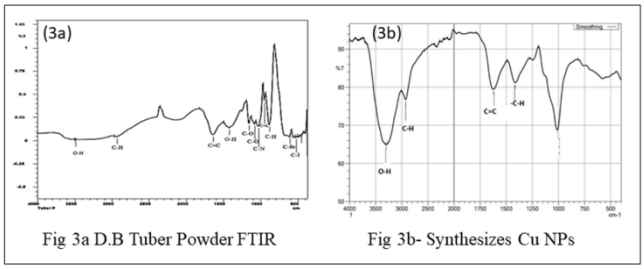


Fig 3a D.B Tuber Powder FTIR Fig 3b- Synthesizes Cu NPs

4] (FESEM)-EADX studies -

In figure 4 (A-D): The synthesized Cu NPs seems to show a cubic and hexagonal agglomerates surface morphology analyzed by Field Emission Scanning Electron Microscopy(FESEM). The Cu element presence also shown by elemental mapping as shwon in the figure 4 (E). The Energy Dispersive X-ray(EDAX) analysis show a signal corresponding to a significant presence of Cu element without any impurity peak as shown in figure 4 (F).

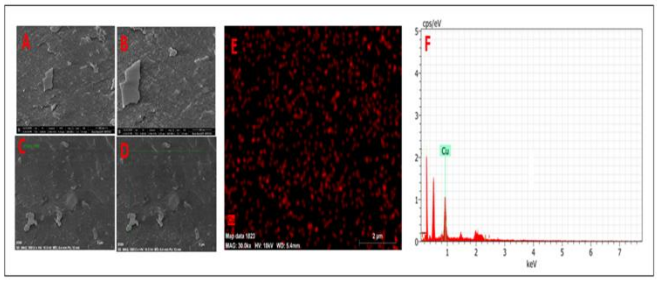


Fig 4(A-D): FESEM of CuNPs Fig 4(E): EDAX Mapping of CuNPs Fig 4 (F) EDAX Pattern ofCuNPs

[5] Antibiofilm Activity –

The schematics of biofilm formation of Candida albicans as shown in the figure 5 with various stages (i) culture medium surface with an adsorbed film of host proteins (blue colour). Initial yeast (red) contact the surface and adhesion to it. (ii) formation of the basal layers with micro colonies. (iii) completion of micro colony formation by addition of the upper and hyphal layer with extracellular matrix material (black) was formed. (iv) mature biofilms contain number of micro colonies with extracellular matrix material that surrounds both yeasts and hyphae (red). The two distinct layers such as a thin and basal region of densely packed yeast cells and an overlying thicker as well as more open hyphal layer. (v) mature biofilms which produces new spores (green) and disperse them [22].

The synthesized Cu NPs are explored for Candida albicans biofilms inhibition. CuNPs shows inhibition as shown in the in Figure 5 (b), 15 × 105 cells/mL were added 15.6 µg/mL to each well of the 96-well polystyrene plates. Similarly, 20 × 105 cells/mL were added 62.5 µg/mL to each well of the 96-well polystyrene plates. It shows maximum inhibition about 20% of biofilm also it slow down the biofilm formation which may various applications in biomedical field [23].

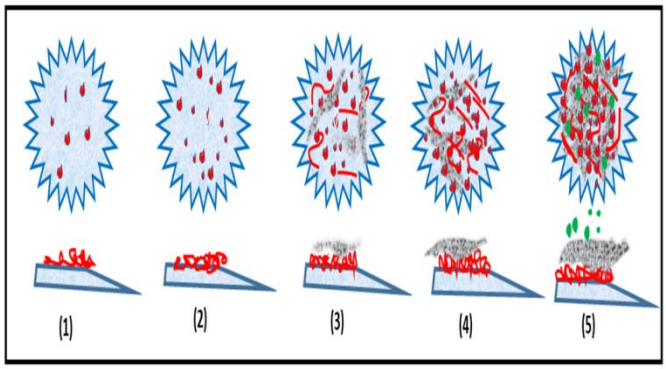


Fig 5(a): Stages in the formation of Candida albicans biofilm.

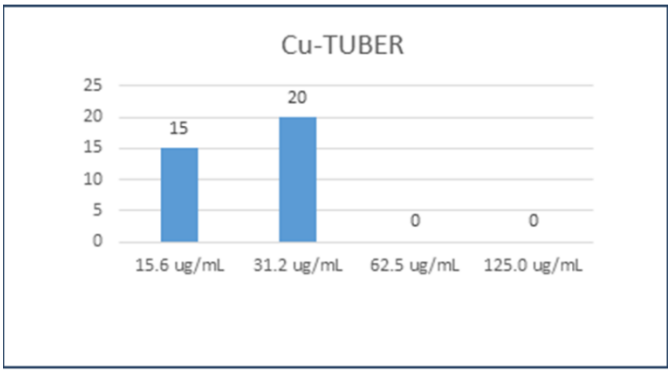


Fig 5(b) Inhibition of Cu NPs

IV. CONCLUSION

Green synthesis approach is an ecofriendly, non-toxic, facile and rapid approach. In present study we synthesized Cu NPs by plant extract Dioscorea bulbifera tuber. X-ray diffraction (XRD) analysis reveals that confirmation of the face centered cubic (FCC) crystal structure without any impurity peaks. UV-visible spectroscopy reveals a strong surface plasmon resonance absorption peak at 570 nm, which attributed to the formation of Cu NPs.

Fourier Transform Infrared Spectroscopy (FTIR) shows the components like flavonoid and phenol were observed, which have contributed as surface capping agents for NPs.Field emission scanning electron microscope (FE-SEM) exhibit a spherical shape of Cu NPs agglomerate morphology and Energy Dispersive X-ray analysis (EDAX) which confirm the presence of only Cu element without any impurities. Cu NPs are used for inhibition of fungal biofilm of Candida albicans which shows about 20 % reduction in biofilm. Cu NPs can be investigating for many biomedical activities such as antibacterial, antioxidant, analgesic, anti-inflammatory, antitumor activities and antidiabetic etc.

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