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RAPID SINGLE STEP GREEN SYNTHESIS OF COPPER OXIDE NANOPARTICLES FROM *VIGNA RADIATA* USING THREE COPPER SALTS AND STUDY ITS ANTIMICROBIAL NATURE

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ABSTRACT

18-50nm sized copper oxide nanoparticle has been synthesized by an ecofriendly method where seed electrolyte of *Vigna radiata* act as a phytoreducer to reduce three different copper salts separately [copper (nitrate, sulphate, chloride)]. Fastest reaction followed by smallest nanoparticle was obtained when electrolyte reacted with copper nitrate. Morphology and charge distribution of nanoparticle was confirmed by characterization and to indentify different phytochemicals which are present in electrolyte, different phytochemical assays were performed by standard techniques. This experiment showed that phenols and flavonoids present in the electrolyte are the main phytoreducer to generate negatively charged copper oxide nanoparticle. Also nanoparticles showed antibacterial activity against *S.aureus*. So the experiment specified that nanoparticle could be generated by a low cost, nontoxic, ecofriendly method which could be further applied in different aspects of pharma industry to ameliorate living standard and health care.

Keywords: *Vigna radiata*; Nanoparticle; Spectroscopy; Phytochemical; Antibacterial activity

INTRODUCTION

Semiconducting nanostructured copper oxide frequently used in solar energy conversion and also used as antibacterial and antifouling agents.[1,2]Complicated, expensive methods are available for synthesis [3]but green synthesis [4,5,6] is far superior to all other techniques. Microbes have risk of contamination but plant extract as a reducer has no such risk. Considering the above characteristics, three different salts of copper, namely copper nitrate, copper sulphate, copper chloride, were reduced by seed electrolytes of *Vigna radiate* to generate copper oxide nanoparticle (CONP) which was applied to *S.aureus* and gave quite satisfactory result for gram positive strain.

MATERIALS AND METHODS

***Materials*:** Chemicals were purchased from MERCK SPECIALITIES Pvt Ltd, Worli Mumbai-400018 and used without further purification. *Vigna radiate* seeds were obtained from the local market. Peptone, beef extract powder, yeast extract powder, sodium chloride, agar–agar were obtained from HIMEDIA LABORATORIES Pvt. Ltd, Mumbai-400086.Double distilled water (DDW) was used throughout the experiment.

CONP synthesis: 100-120 seeds of *Vigna radiata* were surface sterilized and immersed in 150 ml of DDW. After 24 hr, electrolyte of seeds was filtered through a Whatman filter paper No.1. 40 ml of filtered electrolyte was then added individually to three of each conical containing 8 ml of 0.01 M

aqueous solution of copper nitrate and copper sulphate and copper chloride, placing them in a water bath at a constant temperature of 40^{o}C. As the reaction got started partial blue color of solution disappeared and a dark brown color was observed.

Characterization of CONP: Constant monitoring of reduction was done by using UV-Vis spectrophotometer (Perkin –Elmer Lambda 25) against a blank (water).Particle morphology, dispersity and size was measured by dynamic light scattering (DLS-Malvern Nano ZS90)and particle surface charge distribution was obtained through zeta potential (Malvern Zeta Sizer). After that the total solution of CONP was centrifuged at 7000 rpm and repeatedly washed with DDW. Pellet was dried and purified, dried powder was collected and used for Fourier transformed infrared spectroscopy (FTIR-8400S model Shimadzu) analysis. Sample was mixed with potassium bromide (KBr) in 1:100 ratio and spectra were taken. Field emission scanning electron microscopy (FESEM) was done with gold coating for conforming morphology and size by using INSPECT F50 SEM, FEI Europe BV.

Phytochemical Analysis: Calculation of the total phenol, flavonoid, ascorbic acid, reducing sugar and starch, content of *Vigna radiate* seed electrolyte was analyzed by following standard protocol. [7]

Antibacterial activity of CONP: Using the spread plate technique *Staphylococcus aureus* sub sp. aureus (MTCC no.96) (gram positive) and *Escherichia coli* DH5 alpha (MTCC no.1652) (gram negative) were treated with CONP and electrolyte. The strains were grown in bacterial nutrient broth (peptone 0.5%, beef extract 0.1%, yeast extract 0.2%, NaCl 0.5%) for 24hour at 37 ºC then sub-cultured with various concentration of CONP and electrolyte for 24hour.Bacteria (20µl double diluted) were then spread over nutrient agar plate (nutrient broth with agar 1.8% as solidifying agent).After overnight growth, colonies were observed and compared against control plates of both the strains.

Statistical analysis: Analytical determinations were made in three sets of values of all phytochemicals. All experimental data were expressed as mean ± standard deviation. All the statistical analyses were carried out by using Origin software (version Pro 8.5; Origin Lab Corporation, Northampton, MA 01060, USA).

RESULT AND DISCUSSION

Absorption spectra of CONP synthesized from three different copper sources were given in Fig.1 [I]. For all the cases the peak was in between 265-270 nm which is the characteristic peak of CONP [8], while the peak at 340 nm corresponds to raw plant extract. This peak gradually disappeared due to formation of CONP.Some flavonoids and also some phenols has absorption peak at 340nm. [9, 10]Our phytochemical analysis (Table1) showed that the plant extract contained flavonoids and phenol and many more compounds. So it may be concluded that flavonoids and phenols acted as bioreducer. The FTIR spectral Fig 1[II] band at 1599 cm^{-1} indicated the synthesis of CONP. [11]By analyzing the peak position it can be said that aliphatic primary amine N-H stretching, hydroxyl group H-bonded OH stretching and normal polymeric OH stretching can be identified by strong peaks at (3427, 3464, 3400) cm^{-1}.[12] CO_2 stretching represented by 2331 and 2319 is due to the presence of $CuSO_4$ and $CuNO_3$ derived CONP which is absent in case of $CuCl_2$. [13]]Peaks at 1380, 1080,700 cm^{-1} for CONP derived from copper nitrate, copper sulphate, copper chloride which was due to nitrate, sulphate and chloro group present in them.[12]Bands at 1246 cm^{-}1, 1019, 682 and 755 cm^{-1}were represented for skeletal vibration of C-C bond, aliphatic phosphate stretch, aryl thioether stretching and Si-CH_3compound. [12, 14]

Morphological analysis of CONP were observed from FESEM images [Fig.2] which showed that the CONP appeared to have more or less spherical shape with diameter 30-35 nm in case of $CuCl_2$(Fig.2[I]). However, size was 25-50 nm in case of CONP derived from $CuSO_4$ having multifarious shaped (Fig 2 [II]) and size was smallest ie,18-40 nm in case of CONP derived from $CuNo_3$ when used as copper source (Fig2 [III]). Though the prepared nanoparticles were locally clustered but in higher magnification their crystallinity was prominent.

The above results were somewhat supported by the DLS data. The zeta potential of CONP derived from copper nitrate is -12.2mV, followed by -8.45 mV and -6.39 mV for CONP derived from copper sulphate and copper chloride respectively.

As electro kinetic potential is the indicator of stability so we can make a comparison of stability of CONP derived from different salts in the increasing order i.e., copper nitrate>copper sulphate>copper chloride. Smaller the particle sizes higher the zeta potential which confer stability and particles have less chances

of aggregation. So when potential is small, attractive force may exceed this repulsion and get unstable.

Antibacterial study showed in Figure 3A, B, C & D. Best results were obtained when copper nitrate derived CONP (200 µg) and electrolyte (100 ul) were used against *S. aureus* sub sp. aureus (MTCC no.96) (D).Copper ion has antimicrobial property when is in contact with microbes (contact inhibition). [15] There are many possibilities of killing bacteria by CONP as elevated copper ion inside the cell creates oxidative stress, disrupts the DNA and protein chain which ultimately damage the bacterial cell. The lipo-polysaccharide layer (LPS) protects the microbe from copper ion perhaps for this reason gram negative didn't show any sensitivity towards CONP. Electrolyte has secondary metabolites so they also played a vital role in killing.

CONCLUSION

The proposed work can be concluded that bioreduction of CONP was proceed through seed electrolyte in a rapid, ecofriendly, inexpensive single step way from any of the copper salts as mentioned above. Along with environmentally benign synthesis of CONP, antibacterial study showed successful utility of CONP on *S.aureus* as antimicrobial agent and also in therapeutic management.

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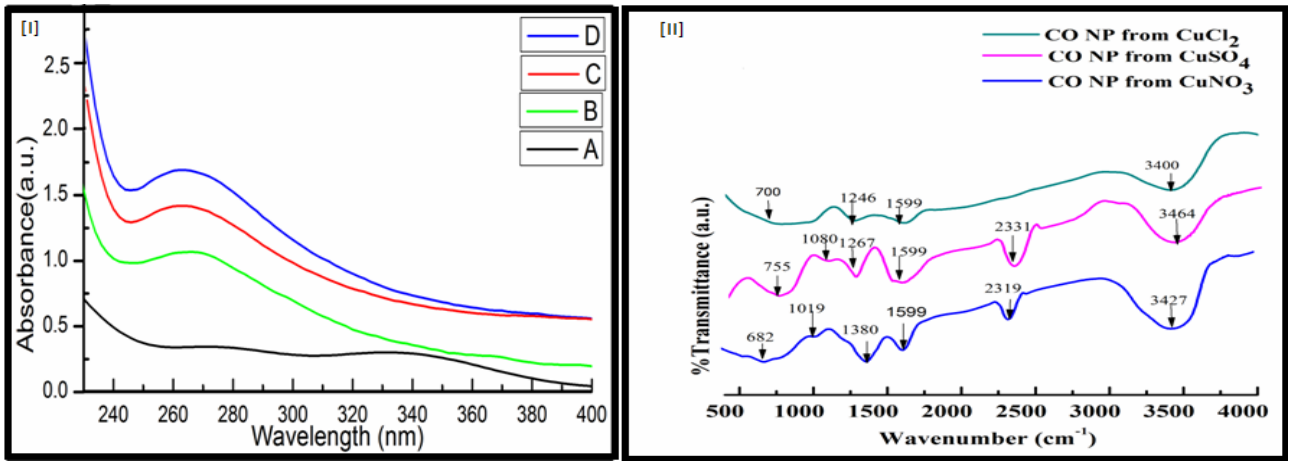

Figure 1: (I) UV-Vis absorbance- A. Raw plant electrolyte; B.CONP derived from $CuNO_3$; C. CONP derived from $CuSO_4$; D. CONP derived from $CuCl_2$. (II)FTIR Spectra of CONP

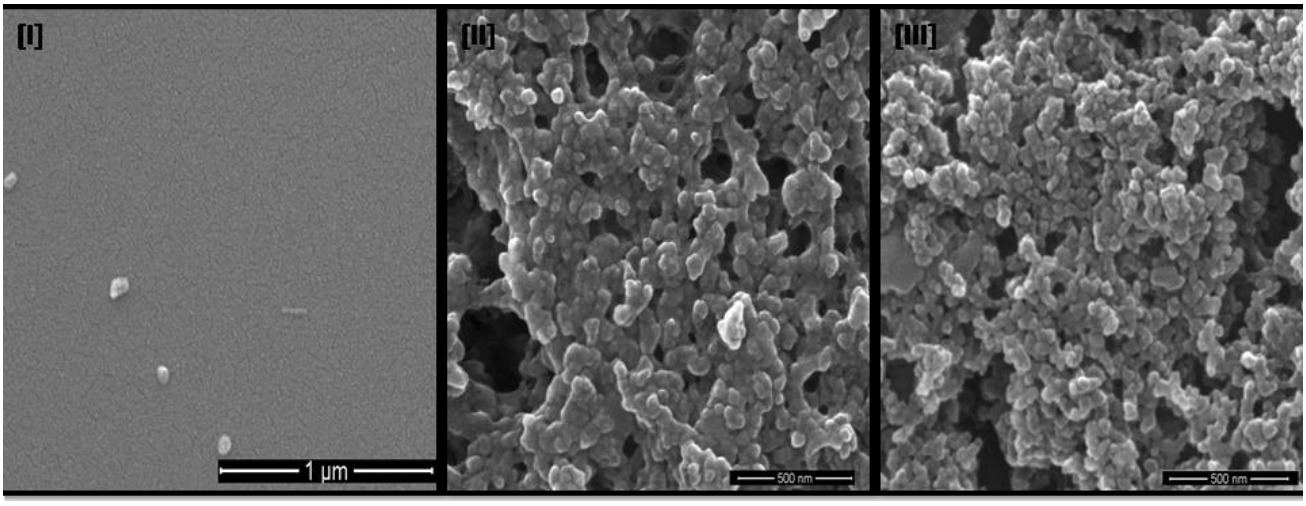

Figure 2: SEM images of CONP :(I)CONP derived from $CuCl_2$, (II)CONP derived from $CuSO_4$, (III) CONP derived from $CuNO_3$

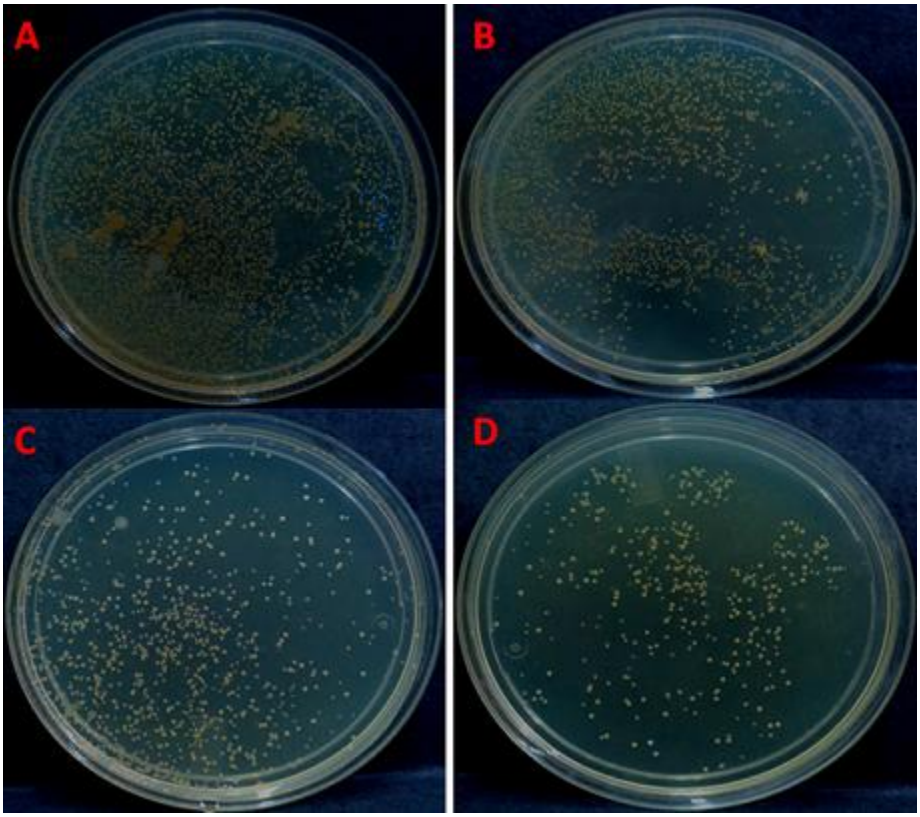

Figure3:Antimicrobial activity of CONP for gram positive bacteria.(A)Control-No plant electrolyte &CONP.(B)Treatment with plant electrolyte & CONP derived from $CuCl_2$(C)Treatment with plant electrolyte & CONP derived from $CuSO_4$,(D)Treatment with plant electrolyte & CONP derived from $CuNO_3$

Table 1: **Phytochemical analysis of *Vigna radiate***

CONTENTS	AMOUNT IN mg/ml(*)
Flavonoids	4.70 ± 0.02
Ascorbic acid	7.20 ± 0.02
Phenols	13.054 ± 0.005
Starch	268.26± 3.71
Reducing sugar	281.66 ± 3.05

* ***Values are means ± standard deviation* n=3**

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