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GREEN SYNTHESIS OF COPPER NANOPARTICLES FROM THE AQUEOUS FRUIT EXTRACT OF *Capsicum annuum* AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

The study highlights the synthesis of copper nanoparticles using aqueous fruit extract of *Capsicum annuum* and its antimicrobial potential. Copper nanoparticles were bio synthesized from copper sulfate penta-hydrate solution ($CuSO4.5H_2O$) with *Capsicum annum* aqueous fruit extract. The obtained copper nanoparticles were characterised by UV-Vis spectroscopy. Scanning electron microscopy revealed the presence of spherical to undefined shape of nanoparticles and particle sizes were found to be in the range of 50 -60 nm. X-ray diffraction spectrum characteristic diffraction peaks were observed at 2 θ range. The Fourier Transform Infrared Spectroscopy reading revealed a number of peaks which correspond to particular functional groups. The results serve potential evidences of the green mediated copper nanoparticles and its antimicrobial activity against *Bacillus subtilis* and *E. coli*.

INTRODUCTION

Capsicum annuum var. *Grossum* commonly known as bell peppers (Solanaceae) is one of the most popular and nutritious vegetable. They are native to Mexico but are cultivated in China, India and other countries (Kuldeep *et al*., 2022). They are one of the oldest cultivated crops which offer flavor, color and add to taste in different food preparations. *Capsicum* is a good source of multiple vitamins. The fruit has been a consumed as a spice by humans and also used in folk medicine in different parts of the world (Meghvansi *et al*., 2010). Capsaicinoids, a group of alkaloids are present in *Capsicum*, which are known for its therapeutic properties (Romero-Luna *et al*., 2022). These alkaloids are used as a counter irritant in neuralgia, rheumatic disorders, lumbago and hay fever. The derivatives of capsaicin are topically applied to cure chronic discomfort syndromes, musculoskeletal pain and diabetes. Capsaicin may also induce burning pain and neurogenic inflammation when used topically (Batiha *et al*., 2020). *Capsicum* fruit extracts contains a number of biomolecules like proteins, enzymes, polysaccharides and vitamins (Zuniga *et al*., 2002). Capsicums are also noted for their anti-aging and cognitive enhancing properties (Ogunruku *et al.,* 2017).

Nanoparticles are unique small particles with distinctive physical, chemical and biological properties. Nanoparticles provide a tremendous driving force for diffusion due to their small size and high surface area to volume ratio. Solid lipid nanoparticles are the novel nano particulate systems that are known to improve the stability of pharmaceuticals (Trilochan and Prasanna, 2013; Mukhrjee *et al*., 2019; Ashok *et al*., 2021; Amica *et al*., 2022). Several approaches such as electrochemical reduction, chemical vapour deposition, thermal decomposition, solvo thermal and chemical reduction have been employed to synthesize metallic nanoparticles. But these approaches consume lot of energy and use toxic solvents leading to generation of hazardous by-products. Green nanotechnology is the alternative solution to decrease the hazardous effects of the production and application of nanomaterials. Eco friendly methods such as catalytic potential (Dahl *et al*., 2007) electrical conductivity (Bonatto *et al*., 2014), optical sensitivity (Quester *et al.,* 2013), magnetic behaviour (Yehia *et al*., 2014) or biological reactivity (Jeetkar *et al*., 2022) are used to characterize the various properties of nanomaterials.

Many plants have been used in the past for the synthesis of metallic and oxide nanoparticles (Jain, 2009; Kirubai *et al*., 2016; Riazunnisa, 2023). Cu nanoparticles have been successfully synthesized from different parts of plant species. Significant pharmacological properties were observed in the Cu nanoparticles extracted from the peel of *Punica granatum* (Kaur *et al*., 2016), juice of *Ziziphus spina-christi* (L.)Willd (Khani *et al*., 2018), root and leaves of *Asparagus adscendens* (Thakur *et al*., 2018), leaves of *Terminalia catappa, Plantago asiatica* and *Areca catechu* (Muthulakshmi *et al*., 2017; Nasrollahzadeh *et al*., 2017; Shwetha *et al*., 2021). The extraction and characterization of copper nanoparticles from plant extracts are reported in number of plants (Sartaj *et al.*, 2023).

Capsicum fruits are known to possess substantial antimicrobial properties (Ekom *et al.*, 2021; Koffi-Nevry *et al.*, 2012). Copper is reportedly known to decrease the microbial concentration by 99.9% (Krithiga *et al.*, 2013). Copper nanoparticles are reported to exhibit broad antimicrobial activity. CuNPs synthesized from plant based extracts interact with bacterial cell membrane disrupting the membrane integrity resulting in the death of the organism (Beyth *et al.*, 2015). Nanoparticles has been synthesized and evaluated for their several properties and applications by many workers to prove its significances (Bhokare *et al.*, 2019). Evidences of green synthesis of silver and gold nanoparticles from the leaf and pulp extract of *Capsicum annuum* are reported (Shikuo *et al.*, 2007; Chun-Gang *et al.*, 2017). According to literature there has not been sufficient attempts to use aqueous fruit extract of *Capsicum annuum* for synthesis of copper nanoparticles. This study focuses on the synthesis and characterization of copper nanoparticles and their antimicrobial properties.

MATERIALS AND METHODS

Plant Collection and preparation of plant extract

Fresh red bell peppers were procured from the local market from Bengaluru, India. The fruit extract of *C. annuum* was used for the green synthesis of CuNPs. The fruits were washed with sterile water, de-seeded and cut into small pieces. 150 gm of the fruits were weighed, homogenized with the help of mortar and pestle and double filtered using Whattman's No.1 filter paper. The filtrate was then centrifuged at 5000 rpm for 15 minutes. The supernatant obtained was collected in a clean conical flask used for experimental work (Tan *et al.*, 2003).

Synthesis of plant mediated copper nanoparticles

100 ml of freshly prepared 0.1M $CuSO_4$. $5H_2O$ was taken in 250 ml conical flaskand kept in the water bath maintained at 80 ºC for 1hr. 100ml of filtered *C. annuum* extracts was then gradually added with constant stirring until the colour of the mixture changed from green to dark brown indicating the formation of CuNPs. The resultant solution was incubated at room temperature for 24hr for the reaction to be completed. The mixture of CuNPs was further centrifuged at 10,000rpm for 10min. Pellet obtained was washed with deionized water. The centrifugation process was repeatedly done to ensure better separation of CuNPs. The pellets obtained were then dried in hot air oven at 50ºC for 24hrs (Batool *et al.*, 2022).

Characterization of synthesized nanoparticles

UV-Visible spectra analysis

A small aliquot of the sample was diluted in distilled water and subjected to spectral analysis using UV-Visible spectrophotometer at the wavelength range of 340-700nm (Wu *et al.*, 2020). The conversion of copper ions to copper nanoparticles was observed by measuring the UV-Vis spectrum.

FT-IR Analysis

FT-IR analysis of the synthesized CuNPs were investigated to evaluate the presence of functional biomolecules or identification of chemical bonding in CuNPs.The discs werescanned in the range of 500-4000cm^{-1} to obtain the spectra.

X-Ray Diffraction (XRD) Measurements

To assess the purity of the degree of crystallization, XRD analysis was carried out. The colloidal suspension containing metal nanoparticle was dried on a small glass slide and was used for XRD pattern analysis (Rajesh *et al.*, 2019). Debye-Scherer's equation was used to calculate the size of copper nanoparticles.

Scanning Electron Microscopy and EDX analysis of copper nanoparticles

The filtrate embedded with copper nanoparticles was dried under vacuum and subjected to SEM studies. This study provided information about the surface of the nanoparticle and its internal structure. Elemental composition of the sample was determined by EDX.

Antimicrobial activity of synthesized nanoparticles

The effect of copper nanoparticles against *Bacillus subtilis* (MTCCID-441) and *Escherichia coli* DH5 (MTCCID-483) bacterial tests trains and *Aspergillus oryzae* (MTCC ID-1846) fungal test strain was determined using agar well diffusion method. Bacterial test organisms were spread over the Muller-Hinton agar (MHA) plates using spread plate method. Similarly fungal spore cultures were spread onto the Potato Dextrose agar (PDA) plates to obtain a microbial lawn. Wells of 3 mm in diameter were cut using a sterile well puncture, which was then loaded with different concentration of 30 μl of CuNPs reconstituted in dimethyl sulphoxide (12.5, 25, 50, 75 ìg of CuNPs per ml of DMSO). Dimethyl sulphoxide was used as negative control. The plates were then incubated and the inhibition zones were measured.

RESULTS AND DISCUSSION

UV-Vis Absorbance Studies

The characteristic appearance of a brown colour, was an indication of the formation of copper nanoparticles in the reaction mixture. The intensity of the brown colour increased during the period of incubation. Similar result was observed from copper nanoparticles synthesized from *Syzygium alternifolium* (Wt.)Walp extract (Yugandhar. *et al., 2017*). Phytochemicals play a major role in first reducing the metal ions and then stabilizing the metal's nuclei in the form of

Table1.Vibrational frequencies of functional groups of possible phyto-constituents obtained by FT-IR analysis

Sl. No.	Frequency (cm^{-1})	Possible functional groups
1	3283.78	O-H stretch of alcohol/phenol
2	2939.18	N-H bend of primary amine
3	2858.1	C-H stretching
4	1736.48	C=O stretching
5	1628.37	C=C stretching
6	1540.54	N-O vibration of nitro compound
7	1445.94	Aromatic C=C stretching derived from aromatic rings
8	1398.64	Symmetric stretching of C-OH
9	1236.48	C-O asymmetric stretching
10	1148.64	C-N stretching vibrations
11	1027.02	Aromatic compounds

nanoparticles.

Polyphenolic compounds such as quercetin, catechin, luteolin are present in *C.annuum* var. *grossum*. The spectrum of the CuNPs dispersion showed a single SPR absorption band with a maximum of approximately 585nm (Figure 1). The spherical CuNPs contribute to surface plasmon resonance (SPR) absorption bands around 500-600nm (Aminu *et al.*, 2019). The position of plasmon absorption peak depends on several factors such as the particle size, shape, type of solvent, *etc.* Variations of the SPR and λ_{max} may be influenced by the structural changes of phytochemicals adsorbed on the nano

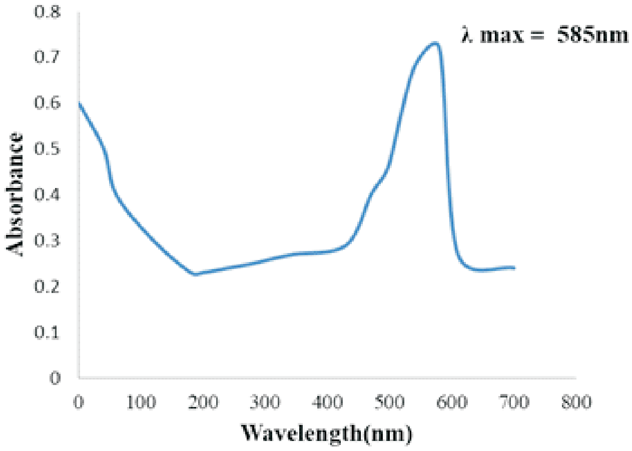

Figure 1.UV-Vis absorption spectrum of *C. Annuum* synthesized CuNPs

surface (Amaliyah *et al.*, 2020)

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra showed a number of absorption peaks (Figure 2; Table 1). The observed difference in the FTIR spectrum of different compounds is traceable to unique arrangement of atoms. The peaks corresponding to O-H,N-H, C=C, C-N,C=O,C-H, are the prominent peaks associated with CuNPs. The peak 3283.78 is characteristic to O-H hydroxyl functional group in alcohol and phenols, while the peak at 2939.18 corresponds to N-H bend of primary amine. The peak at 1445.94 corresponds to the aromatic C=C stretching derived from aromatic rings and the peak at 1236.48

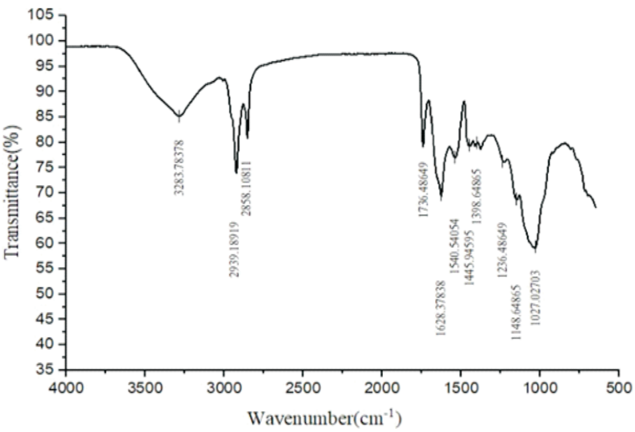

Figure 2. FTIR spectra of biosynthesized copper nanoparticles

corresponds to C-O asymmetric stretching. Similar results were observed in the other plant extracts. These bio-entities are known to fabricate and stabilize the copper nanoparticles in the aqueous solution (Kuppusamy *et al.,* 2017; Lalitha *et al.*, 2022).

X-ray diffraction (XRD)

X-ray diffraction (XRD) studies revealed the crystalline nature of the copper nanoparticles. At 2θ value, a number of Bragg reflection peaks were observed at 43.31, 50.45, 74.09 and90.6, which were indexed to (111), (200), (202), and (311) crystallographic planes of face-centered cubic (Figure 3). The estimated mean particle size was 51nm (calculated using Debye-Scherer's equation). The small size of the nanoparticles synthesized thus increases their high surface area, and surface

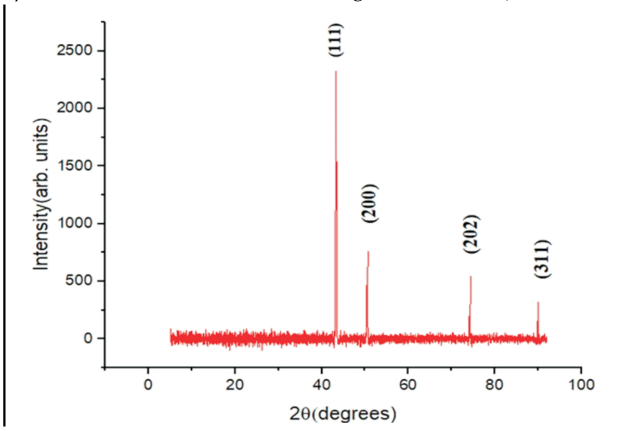

Figure 3. XRD analysis of copper nanoparticles

area to volume ratio. These XRD results were similar to previous literature (Shailesh *et al.*, 2018; Sartaj *et al.*, 2023).

SEM-EDX Analysis

Results of SEM and EDX revealed that most of the synthesized copper nanoparticles were spherical with an average particle size of 50-60nm along with a number of aggregates, while some of the nanoparticles had an undefined shape. EDX analysis revealed the purity of synthesized CuNPs (Figure 4 and 5 respectively) (Mali *et al.*, 2019). Oxygen with copper in EDX spectrum indicates the presence of copper in oxide or dioxide form. *C. annuum* mediated CuNPs showed 40.37% purity for Cu along with few weak signals of C, O, S, K and P

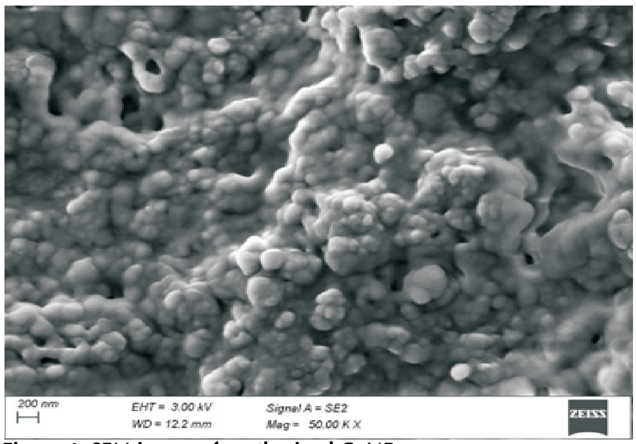

Figure 4. SEM image of synthesized CuNPs

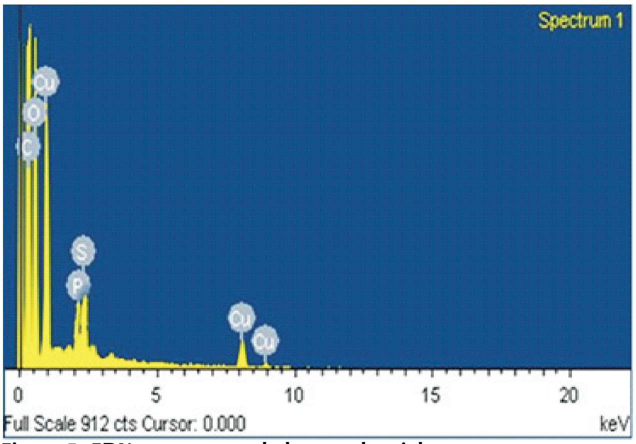

Figure 5. EDX spectrum and elemental weight

element owing to interactions with extract during bioprocessing. Phyto-constituents such as like flavonoids, phenolic compounds, carbohydrates, glycosides, steroids and tannins present in the plant extract may also produce some weak signals due to the X-ray emissions (Mali *et al.*, 2020).

Antimicrobial activity of copper nanoparticles

Significant results were observed on subjecting the synthesized green CUNPs to antimicrobial studies. The copper nanoparticles were effective against *Bacillus subtilis* and *E. coli* (Khan and Mateen, 2018). Maximum zone of inhibition of 19.5 mm and 15.1 mm was obtained at a concentration of 75μg/ml respectively (Table 2; Figure 7 and 8). These results are in accordance with the earlier work on gram positive and negative bacteria (Casimir *et al.,* 2018). A significant decrease

Table 2. Antimicrobial activity of synthesised nanoparticles from *C. annuum* var. *grossum*. Values represent mean ± SD

	Zone of Inhibition (mm)		
Concentration (¼g/ml)	*E.coli*	*Bacillus subtilis*	*Aspergillus oryzae*
75	15.1±0.28	19.5±0.5	10.6±0.57
50	13.6±0.57	17.6±0.57	8.8±0.28
25	10.6±0.5	14.0±0.0	5.8±0.28
12.5	5.6±0.28	9.5±0.5	2.83±0.28
Positive control	4.2±0.5	3.5±0.5	8.0±0.0

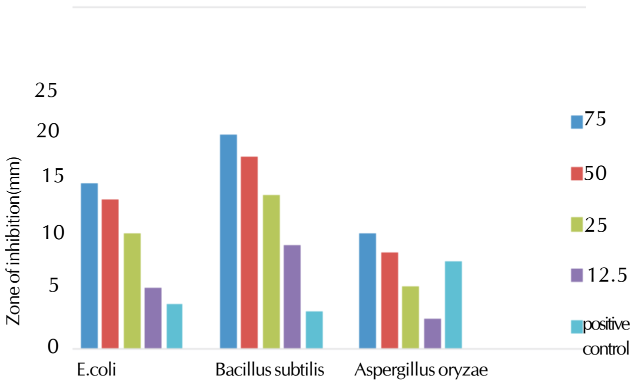

Figure 7. Antimicrobial activity of CuNPs from *C. annuum* var. *Grossum*

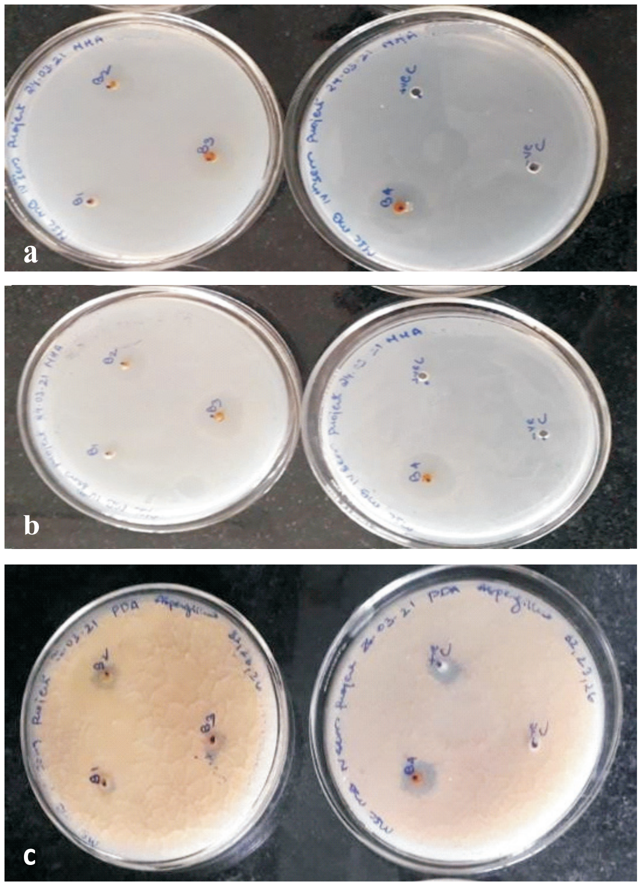

Figure 8. Antimicrobial activity of CuNPs from *C. annuum* var. *Grossum*

a – *E.coli*; b – *Bacillus subtilis*; c – *Aspergillus oryzae*

in microbial growth was observed with increase in the concentration of CuNPs. The effect of CuNPs on fungi were comparatively insignificant probably due to the fungal cell wall architecture and composition. The firmness of the fungal cell wall is due to the presence of chitin, and also contributed by the polysaccharides, thereby, not allowing easy passage of CuNPs from the outer layer of the cell wall to the inner layer. The copper nanoparticles destroy the enzymes of microbial cell membrane and act as inhibitory substances (Ren *et al.*, 2009). The effective use of copper metal as antimicrobial agent is well known, although the exact mechanism of action of CuNPs needs to be probed further. There could be several sources that contribute to the release of copper ions, their permeation and breakdown of cellular membranes and influence the biochemical pathways (Babushkina *et al.*, 2015). Capsicum fruits are known to contain sufficient capsaicinoids and phenolic compounds that are responsible for the inhibition of microbes, through an increase in the permeabilization of the cell membrane and cell wall (Romero-Luna *et al.*, 2022). The synthesized CuNPs, exhibited significant anti-microbial results that affirm the use of *C. annuum* against the diseases caused by the tested pathogens (Koffi-Nevry *et al.,* 2012). The research finding obtained in the present work may provide data for further studies on Capsicum extracts that may be beneficial against pathogenic microorganisms.

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