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ANTIBACTERIAL EFFICACY OF BIOGENIC COPPER NANOPARTICLES SYNTHESIZED FROM *OCIMUM SANCTUM* LEAF EXTRACT

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ABSTRACT: A major challenge in treating bacterial infections is the increasing incidence of microbial resistance to antibiotics. This poses a serious threat prompting the search for alternative strategies to treat bacterial infections. Metal nanoparticles as novel antibiotic agents hold promise because they show strong antibacterial activity against various bacterial species, including Gram-positive and Gram-negative bacteria. Green synthesis of nanoparticles offers minimization of wastage, reduction of derivatives, use of non-toxic solvent, auxiliaries and renewable feedstock. A single step, an eco- friendly, cost-effective method is used for the synthesis of copper nanoparticles (CuNPs) from 1.0 mM copper sulphate solution using extract of *Ocimum sanctum* (Tulsi) leaves as reducing and capping agent. UV-VIS, FESEM, FTIR and XRD were used to confirm and characterize the NPs. The biosynthesized copper NPs were phased pure and well crystalline with a simple cubic structure. Antibacterial study of the biogenic CuNPs suggests their efficacy against common human bacterial pathogen species. Gram-negative test organisms were found to be more susceptible to toxicity of biosynthesized CuNPs. Metal nanoparticles hold the promise to overcome microbial resistance due to their specific properties.

INTRODUCTION: Though metals like copper, silver, and gold have been used as antibacterial agents for centuries, their efficacy is nowhere in comparison to modern antibiotics; consequently their use has diminished. Metals in their nanoparticle form are prevalent in use for the past one and a half decades. The special interest in nanoparticles as antibacterial agents lies in the ability to prepare them with high surface area, crystalline morphologies and potential reactive sites.

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The advent of new chemical and physical methods for the synthesis of nanomaterials has raised the concern for environmental safety as a large amount of hazardous by-products are generated. Thus, there is a need for 'green chemistry or technology. Various unicellular and multicellular organisms are known to produce inorganic materials either intra or extra-cellularly [1] often of nanoscale dimensions and exquisite morphology and hierarchical assembly.

Biosynthesis of nanoparticles by plant leaf extract and their potential applications is well documented by Sastry *et al.* [2] Owing to its wide occurrence in India and great therapeutic potential, the practitioners of traditional systems of medicine have been using *Ocimum sanctum* L. (Tulsi) for curing various ailments. The antibacterial activity of Tulsi was reported by Bartels [3]. Tulsi leaf extract

has been used in the synthesis of silver, gold and copper nanoparticles [4, 5, 6].

MATERIALS AND METHODS:

Bacterial Strains and Media: Representative micro-organisms of Gram-positive bacteria (*Bacillus subtilis* MTCC 441, *S. aureus* MTCC 2940) and Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 9027, *E. coli* ATCC 8739) were used to evaluate the anti-microbial activity of prepared copper nanoparticles.

Bacterial strains were maintained on suitable agar slants. *Pseudomonas aeruginosa*, *S. aureus* were first grown on blood agar at 37 °C and kept at 4 °C. For each experiment, one colony was inoculated in Tryptone Soy Broth, BHI (Brain Heart Infusion) broth respectively and cultured for 16 h.

Tulsi Leaves Extract (TLE) Preparation: *Ocimum sanctum* (Tulsi) leaves were collected from botanical garden Nagpur, Maharashtra. The plant material was identified by comparison with reference material (authentication no. 10308) at the Department of Botany, RTMNU Nagpur. After thorough washing and drying, 10.0 g leaves were ground to a fine paste and boiled in 100.0 ml water for 30 min.

The extract was filtered and subsequently, 1.0 ml of the extract was made to 100 ml with 1.0 mM copper sulphate solution and incubated at 37 °C for 24 h with continuous agitation. A change in color was observed. The suspension was centrifuged at 10,000 rpm for 10 min. The pellet was collected and lyophilized for further studies.

Characterization of Nanoparticles:

UV-Vis Spectra Analysis: The NPs synthesis was monitored using Elico BL 198 Bio spectro-photometer over the spectral range of 200-900 nm using an aliquot of reaction mixture diluted with distilled water.

Field Emission Scanning Electron Microscopy (FESEM): The shape and size of silver nano-particles were determined by FESEM (Field Emission Scanning Electron Microscopy). The micrographs were obtained using Joel JSM -7610 F operating at 80 kV. The film was prepared by coating the nanoparticles with a thin layer of gold to make them more conducive to current.

Fourier Transform Infra-Red spectroscopy (FTIR): FTIR (Fourier transform Infra-Red) spectra of the NPs was recorded over the range of 400-4,000 cm^{-1} on a Perkin Elmer Spectrum one FTIR spectrometer. FTIR measurements were carried out by employing KBr pellet technique. The FTIR spectra were collected from running 12 scans at a resolution of 4 cm^{-1} in the transmission mode.

X-Ray Diffraction Analysis (XRD): The crystalline structure and phase purity of the Cu NPs produced were identified by X-ray diffraction. XRD pattern was obtained using XPert Pro X-ray diffractometer (PANalytical, Japan). The target was Cu (kα) radiation 1.54 A°, the generator operated at 45 kV and 40 mA. The scanning mode was continuous with scanning range 2Φ from 10- 99.

Antibacterial Study: The agar well diffusion assay method was used to assess the antibacterial activity of the biosynthesized Cu NPs. 20.0 ml of molten and cooled suitable agar media was poured in sterilized Petri dishes. The plates were subjected to sterility check by leaving them overnight at room temperature. The bacterial test organisms were grown in suitable media for 24 h and used to prepare bacterial lawns (1×10^5 cfu/ml) by pour plate method. 5.0 mm diameter agar wells were prepared with the help of sterilized steel borer. For the preliminary antibacterial study, wells were loaded with varying concentrations (10, 50, 100, 150 µg/ml) of suspended Cu NPs in water. The plates were incubated at 37 °C for 24 h and examined for the presence of inhibition as a clear area around the wells. The diameter of the zone of inhibition was measured and means value expressed in millimeter. Subsequently, 100 µg/ml NP suspension was used for comparative studies. Chloramphenicol (25 µg/ml), 1.0 mM copper sulphate solution, inoculated media without nanoparticles served as controls.

Statistical Analysis: Experiments were performed in triplicates and data represented as mean with standard deviation.

RESULTS AND DISCUSSION: Many biological systems, including plants, can transform inorganic metal ions into metal nanoparticles *via* reductive capacities of the proteins and metabolites present in them.

Plant extracts have been extensively employed as an efficient resource for the synthesis of materials [7, 8, 9]. The present study concentrated on bio-fabrication of CuNPs as they are shown to possess strong antibacterial capacity [10, 11].

The reduction of Cu ions to Cu particles was followed as a change in color from light yellow to black-brown **Fig. 1**. The surface plasmon resonance phenomenon imparts a brown color to the CuNPs in aqueous solution.

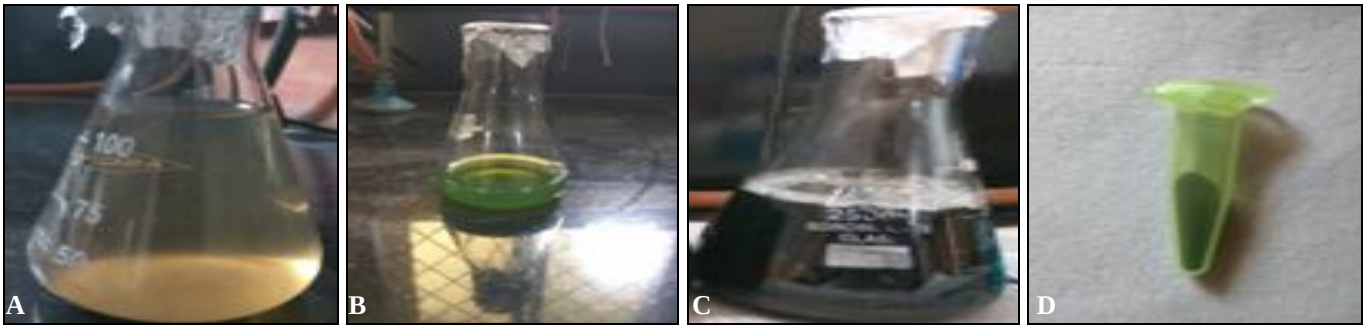

FIG. 1: FABRICATION OF CU NANOPARTICLES A: TULSI LEAVES EXTRACT B: EXTRACT MIXED WITH COPPER SULPHATE SOLUTION C: COLOUR CHANGE AFTER SYNTHESIS OF Cu NANOPARTICLES D: LYOPHILIZED NANOPARTICLES

UV-Visible spectroscopy is useful for the monitoring of organometallic species. UV-Vis spectrum of colloidal solution of CuNPs has been recorded as a function of time. At 3 h, the CuNPs in the reaction mixture demonstrates a distinct peak at 563 nm **Fig. 2**.

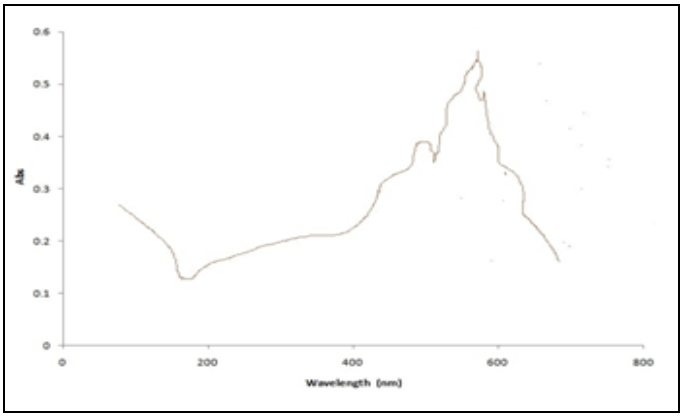

FIG. 2: UV-VIS ABSORPTION SPECTRUM OF CU NANOPARTICLES

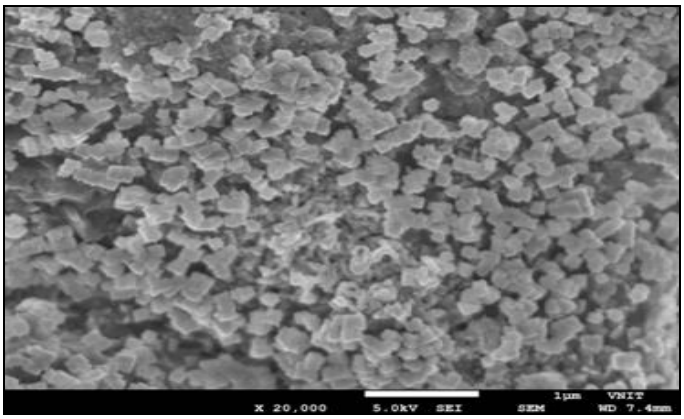

FIG. 3: FIELD SCANNING ELECTRON MICROGRAPH OF BOSYNTHESIZED Cu NANOPARTICLES

Various techniques help in understanding the chemical functionality of the sample. FTIR spectroscopy was used to identify the functional groups of the active components based on the peak value in the region of infrared radiation **Fig. 4**.

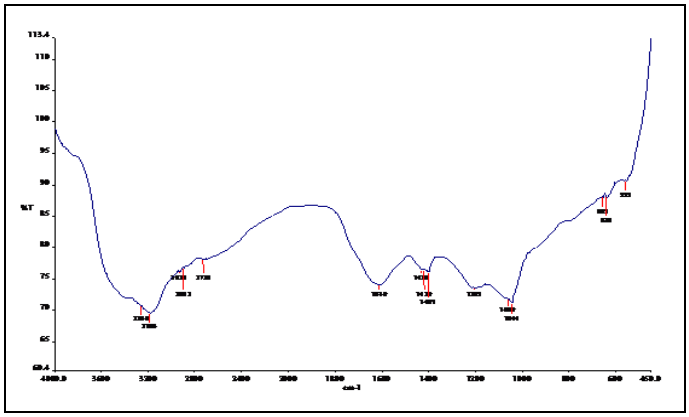

FIG. 4: FOURIER TRANSFORM INFRARED SPECTRUM OF BIOSYNTHESIZED Cu NANOPARTICLES

The FTIR Spectrum of CuNPs showed strong peaks corresponding to different functional groups [12]. The peak at 3260 cm^{-1} can be attributed to –OH group, H bonded –OH stretch, 3186 corresponds to polymeric –OH stretch; 2935, 2893 are due to methylene and methylene C-H stretch respectively. 1610 cm^{-1} arise because of C=C-C aromatic ring stretch and 1401 cm^{-1} is characteristic of phenol or tertiary alcohol –OH bond. Aromatic C-H in-plane bend is responsible for minor peaks 1203-1004 cm^{-1}. The weekly expressed bands at 655, 638 cm^{-1} are due to two main vibrational modes in metal-oxygen (Cu-O) bond, depending on the degree of hydrogen bonding [13]. An essential oil from Tulsi contains Eugenol, methyl chavicol, linalool, caryophyllene oxide, and camphor and beta elemene. Phyto-chemical analysis of Tulsi extract shows that it contains phenylpropanoids, triterpenes, flavonoids and phenolic acids. Plant metabolites such as sugars, terpenoids, polyphenols, alkaloids, phenolic acids and proteins play an important role in the reduction of metal ions to nanoparticles and in supporting their subsequent stability [14, 15, 16]. The large amount of flavonoids and terpenoids present in Tulsi leaves aqueous extract to play an important role in reduction reactions hence it can be hypothesized that eugenol, which is a phenolic compound acts as the principal reducing and capping agent along with epigenin, ursolic acid and rosmarinic acid for biofabrication of CuNPs **Fig. 5**.

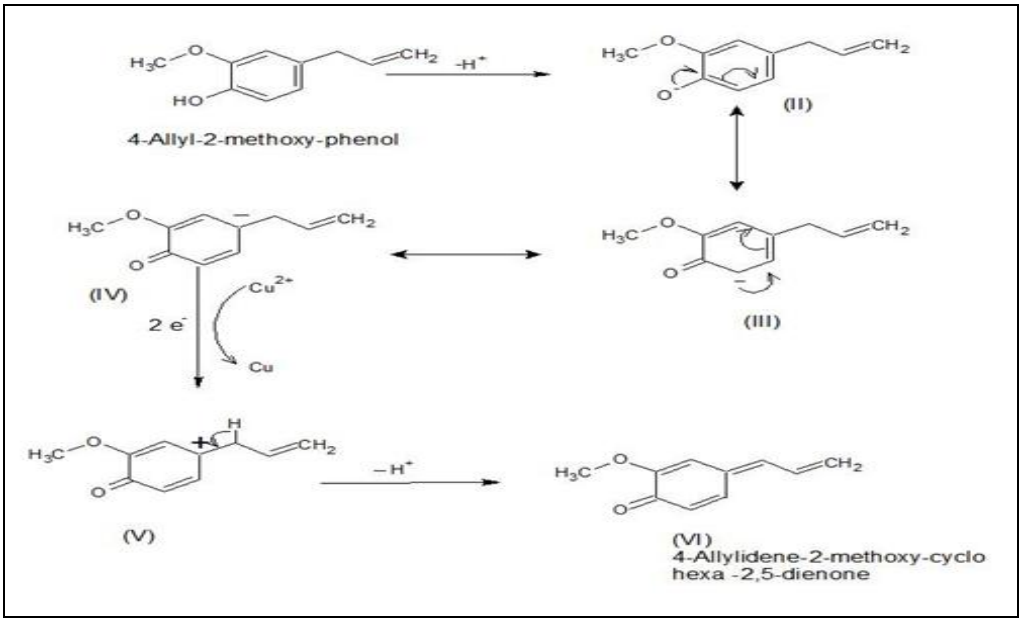

FIG. 5: HYPOTHESIZED MECHANISM OF REDUCTION OF Cu IONS TO METAL BY EUGENOL (4-ALLYL -2-METHOXY- PHENOL)

The dissociation of a proton of the eugenol OH-group results in the formation of resonance structures capable of further oxidation. This process is accompanied by an active reduction of metal ions followed by NP formation. In sweet basil, flavonoids are involved in the initiation of NP formation (nucleation) and further aggregation in addition to the bio-reduction stage. Peaks at 2Ɵ of 34º, 43º, 47º, 72º in the XRD pattern of the biogenic CuNPs suggests presence of (111), (210), (211), (222), (410) facets of a simple cubic crystal (scc) of CuNPs in agreement with JCPD (Joint committee on powder diffraction) **Fig. 6**. Kulkarni 6 reported Cu nanoparticles with fcc geometry. The difference in size and morphology may be attributed to the interactions of the biomolecules present in the leaf extract with metal ions [17, 18, 19].

From X-ray diffraction pattern data the average size of the nanoparticles was found to be 54.31 nm. NPs with anti-bacterial activities have the potential to reduce or eliminate the evolution of more resistant bacteria because they target multiple biomolecules simultaneously avoiding the development of resistant strains. The biofabricated CuNPs are more potent inhibitors of the test organism as compared to the plant extract as shown in **Fig. 7**. This high antibacterial activity of CuNPs could be attributed to their size and specific morphology [20, 21].

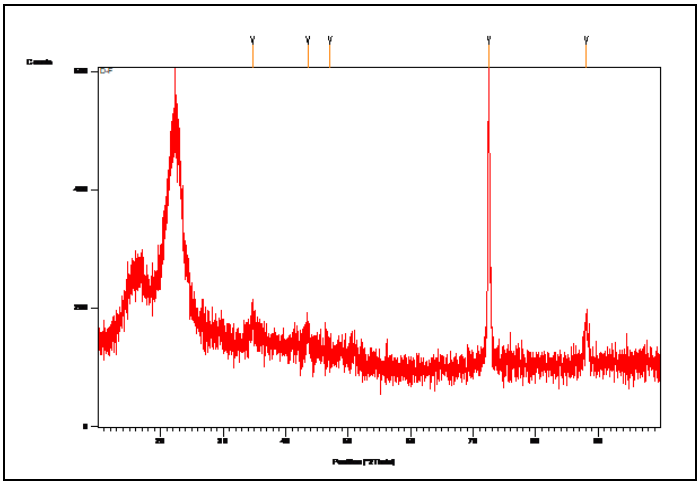

FIG. 6: X-RAY DIFFRACTION PATTERN OF BIOSYNTHESIZED Cu NANOPARTICLES

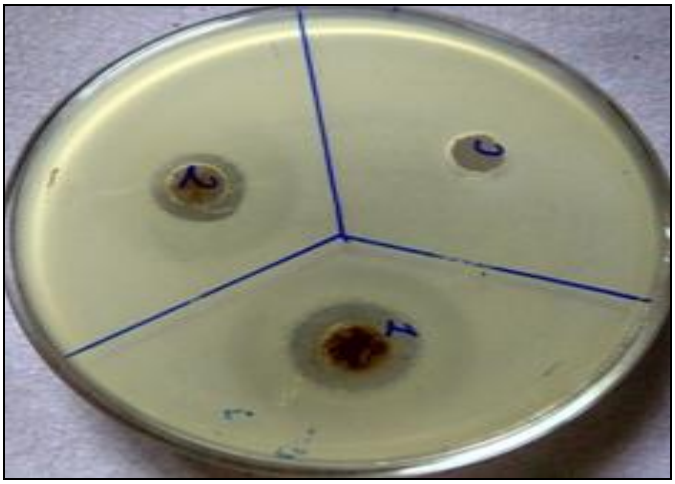

FIG. 7: ANTBCATERIAL ACTIVITY OF BIO-SYNTHESIZED CU NANOPARTICLES AGAINST *P. AERUGINOSA* C: CONTROL 1: CU NANOPARTICLE SUSPENSION 2: LEAVES EXTRACT

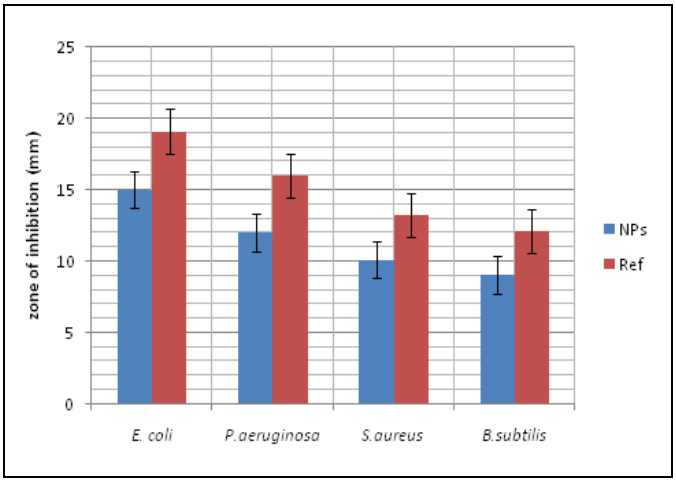

FIG. 8: ANTIBACTERIAL ACTIVITY AGAINST TEST ORGANISMS THIS INHIBITORY ACTIVITY AS REPORTED IS COMPARED WITH STANDARD INHIBITORY DRUG, CHLORAMPHENICOL

The nanoparticles synthesized by green technology were found to be toxic against the four common human bacterial pathogens at a concentration of 100 µg/ml **Fig. 8**.

The concentration of NPs directly affects toxicity because a larger concentration of NPs releases more ions [20, 22] with a concomitant increase over time [23]. Concentration of CuNPs is important as a low concentration of NPs cause a delay in lag phase which suggests a micro-nutritional role of Cu for bacteria. Cu is an essential element playing a role as a co-factor for different enzymatic systems, such as those involved in redo reactions essential to cellular respiration (cytochrome oxidase) and superoxide dismutase (antioxidant defense) [24]. An inhibition in bacterial growth at a high concentration of CuNPs suspension required underlines this essentiality of Cu in physiological systems. CuNPs acts as an antibacterial in multiple ways including adhesion to Gram-negative bacterial cell wall due to electrostatic interactions, having an effect on protein structure in cell membranes, causing denaturation of intracellular proteins and interaction with P and S containing compounds like DNA [25].

In contrast to Ruparelia [26], the higher antibacterial activity of the biogenic CuNPs from Tulsi leaves extract is evident against Gram-negative organisms. The difference in the cell wall structures could be responsible for the phenomenon. In the case of Gram-negative bacteria, such as Escherichia coli, bacterial cells are covered by a layer of lipopolysaccharides (1–3 µm thick) and peptidoglycans (~ 8 nm thick). This arrangement may facilitate the entrance of released ions from NPs into the cell. On the other hand, Gram-positive

bacteria such as *Staphylococcus aureus* possess a peptidoglycan layer much thicker than Gram-negative bacteria, spanning over 80 nm with covalently attached teichoic and teichuronic acids. The cell wall destruction that occurs from the physical interaction between NPs and the cell wall is, therefore, more detrimental for Gram-negative bacteria. Thus, though both Gram-positive and Gram-negative bacteria have a negatively charged cell wall, Gram-positive bacteria are usually more resistant to NP mechanisms of action [27, 28, 29].

The difference in observations could be due to the variables such as materials and methods used for the synthesis of NPs, growth media used for culture of test organisms, concentration and size of the NPs used in the present study [30]. Also, in the Gram-negative bacteria rather than a continuous layer, there are certain focal areas rich in negative charges [31]. These negatively charged molecules have a higher affinity for the positive ions that most of the NPs release, leading to a build-up and increased uptake of ions, which then cause intracellular damage. Thus, a potential binding of a high number of NPs on these anionic domains may enhance the toxicity. Electrophoretic mobility studies and mathematical calculations also demonstrated that *E. coli* is more negatively charged and rigid than *S. aureus* [32].

CONCLUSION: The use of biological matter such as plants for synthesis of nanoparticles offers an alternative, efficient, inexpensive and environmentally safe method for producing nanoparticles with specified properties. Biogenic CuNPs were fabricated from *Ocimum sanctum* (Tulsi) leaves extract using green technology. The synthesis was validated by the UV-Vis spectra of an aqueous solution of the fabricated NPs. FESEM helped in the determination of the structural characteristics of the NPs. Functional groups involved in the reductive process during NP synthesis could be elucidated with FTIR spectroscopy.

A preliminary study suggests significantly higher functional bioactivity (antimicrobial) of Cu-NPs than the bulk material. A major component of the extract, eugenol is hypothesized to play an important role in the synthesis of NPs. The NPs exhibited strong to moderate antibacterial activity against common pathogens which can further be tested against the rapidly growing population of multidrug-resistant pathogens.

It is hoped that fabricated NPs is a better drug delivery system because of their biogenic nature and newer developments in the field will surely help in the treatment of infections without the fear of antibiotic resistance because of NPs target multiple biomolecules simultaneously.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

REFERENCES:

1. Simkiss K and Wilbur KM: Biomineralization. Academic Press New York (1989).
2. Sastry M, Patil V and Sainkar SR: Electrostatically controlled diffusion of carboxylic acid derivatized silver colloidal particles in thermally evaporated fatty amine films. J Phys Chem B 1998; 102: 1404-10.
3. Bartels HA: The effect of eugenol and oil of cloves on the growth of microorganisms. American J Orthodont Oral Surg 1947; 33: 458-65.
4. Philip D and Unni C: Extracellular biosynthesis of gold and silver nanoparticles using Krishna tulsi (*Ocimum sanctum*) leaf. Physica E 2011; 43(7): 1318–22.
5. Dina MI, Arshada F, Rania A, Aihetashamb A, Mukhtarb M and Mehmood HA: Single step green synthesis of stable copper oxide nanoparticles as efficient photo catalyst material. Journal of Optoelectronics and Biomedical Materials 2017; 9: 41-48.
6. Kulkarni VD and Kulkarni PS: Green Synthesis of Copper Nanoparticles Using *Ocimum Sanctum* Leaf Extract. International Journal of Chemical Studies 2013; 1(3): 1-4.
7. Parthibana E, Manivannanb N, Ramanibaia R and Mathivanan N: Green synthesis of silver-nanoparticles from *Annona reticulata* leaves aqueous extract and its mosquito larvicidal and anti-microbial activity on human pathogens. Biotechnology Reports 2018; 20: 1-10.
8. Benakashania F, Allafchian AR and Jalalic SAH: Biosynthesis of silver nanoparticles using *Capparis spinosa* L. Leaf extract and their antibacterial activity. Karbala International Journal of Modern Science 2016; 2: 251-58.
9. Lakshmanan G, Sathiyaseelan A, Kalaichelvan PT and Murugesan K: Plant-mediated synthesis of silver nanoparticles using fruit extract of *Cleome viscosa* L: Assessment of their antibacterial and anticancer activity. Karbala Int Journal of Modern Science 2018; 1: 61-68.
10. Mahmoodi S, Elmi A and Hallaj-Nezhadi S: Copper Nanoparticles as Antibacterial Agents. Molecular Pharma and Organic Process Research 2018; 6(1): 140.
11. Guo J, Gao SH, Lu J, Bond PL, Verstraete W and Yuan Z: Copper oxide nanoparticles induce lysogenic bacteriophage and metal-resistance genes in *Pseudomonas aeruginosa*. PAO1. ACS Appl Mater Interfaces 2017; 9: 22298-307.

12. Colthup N: Introduction to infrared and Raman spectroscopy. Elsevier, Amsterdam 2012.
13. Moniri S, Ghoranneviss M, Hantehzadeh MR and Asadabad MA: Synthesis and optical characterization of copper nanoparticles prepared by laser ablation. Bulletin of Materials Science 2017; 40(1): 37-43.
14. Shiv Shankar S, Ahmad A, Pasricha R and Sastry M: Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. J Mater Chem 2003; 13: 1822-46.
15. Song JY, Kwon EY and Kim BS: Biological synthesis of platinum nanoparticles using Diopyros kaki leaf extract. Bioprocess Biosyst Eng 2010; 33: 159-64.
16. Kulkarni N and Muddapur U: Biosynthesis of metal nanoparticles: a review. J Nanotechnol 2014: 1-8.
17. Patil BN, Taranath TC and Limoniaacidissima L: leaf mediated synthesis of zinc oxide nanoparticles: A potent tool against Mycobacterium tuberculosis. Int J Mycobacteriol 2016; 5(2): 197-04.
18. Nasrollahzadeh M, Sajadi SM, Rostami-Vartooni A and Hussin SM: Green synthesis of CuO nanoparticles using aqueous extract of *Thymus vulgaris* L. leaves and their catalytic performance for N-arylation of indoles and amines. J of Col and Interface Science 2016; 466: 113-19.
19. Shankar SS, Rai A, Ahmad A and Sastry M: Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. J colloid Interface Sci 2004; 275: 496-02.
20. Tamayo LA, Zapata PA, Vejar ND, Azócar MI, Gulppi MA, Zhou X, Thompson GE, Rabagliati FM and Paez MA: Release of silver and copper nanoparticles from polyethylene nanocomposites and their penetration into *Listeria monocytogenes*. Mater Sci EngC 2014; 40: 24-31.
21. Cioffi N, Torsi L, Ditaranto N, Tantillo G, Ghibelli L, Sabbatini L, Bleve-Zacheo T, D'Alessio M, Zambonin PG and Traversa E: Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. Chemistry of Materials 2005; 17(21): 5255-62.
22. Nezhad SS, Khorasgani RM, Emtiazi G, Yaghoobi MM and Shakeri S: Isolation of copper oxide (CuO) nanoparticles resistant Pseudomonas strains from soil and investigation on possible mechanism for resistance. World J Microbiol Biotechnol 2014; 30: 809-17.
23. Rousk J, Ackermann K, Curling SF and Jones DL: Comparative toxicity of nanoparticulate CuO and ZnO to soil bacterial communities. PLoS ONE 2012; 7: e34197.
24. Linder MC and Hazegh-Azam M: Copper biochemistry & molecular biology. Am J Clin Nutr 1996; 63: 797S-811S.
25. Raffi M, Mehrwan S, Bhatti TM, Akhter JI, Hameed A, Yawar W and Hasan MM: Investigations into the antibacterial behavior of copper nanoparticles against *Escherichia coli*. Ann Microbiol 2010; 60: 75-80.
26. Ruparelia JP, Chatterjee AK, Duttagupta SP and Mukherji S: Strainspecificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater 2008; 4: 707-16.
27. Mukha IP, Eremenko AM, Smirnova NP, Mikhienkova AI, Korchak GI, Gorchev VF and Chunikhin A: Antimicrobial activity of stable silver nanoparticles of a certain size. Appl Biochem Microbiol 2013; 49: 199-06.
28. Dorobantu LS, Fallone C, Noble AJ, Veinot J, Ma G, Goss GG and Burell RE: Toxicity of silver nanoparticles against bacteria, yeast and algae. J Nanopart Res 2015; 17: 172.
29. Memic A: Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. The International Journal of Nanomedicine 2012; 7: 6003-09.
30. Kim TH, Kim M, Park HS, Shin US, Gong MS and Kim HW: Size-dependent cellular toxicity of silver nanoparticles. J Biomed Mater Res A 2012; 100: 1033-43.
31. Magnusson KE and Bayer ME: Anionic sites on the envelope of Salmonella typhimurium mapped with cationized ferritin. Cell Biophys 1982; 4: 163-75.
32. Sonohara R, Muramatsu N, Ohshima H and Kondo T: Difference in surface properties between *Escherichia coli* and *Staphylococcus aureus* as revealed by electrophoretic mobility measurements. Biophys Chem 1995; 55: 273-7.

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