***Morinda citrifolia* LEAF EXTRACT MEDIATED GREEN SYNTHESIS OF COPPER OXIDE NANOPARTICLES AND IT'S POTENTIAL AND ANTIBACTERIAL STUDIES**

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ABSTRACT

Eco responsive strategies of green mediate synthesis of nanoparticles are the current analysis within the extremity of nanotechnology. Copper Oxide nanoparticles (CuO Nps) based on *Morinda citrifolia* leaf extract were synthesized by a biological method and characterized by XRD, SEM, UV-DRS, Zeta potential, and FTIR. The high nutritional worth of *Morinda citrifolia* could induce therapeutic effects, together with antimicrobial and antioxidant properties. XRD exhibits an orthorhombic structure. The aspect of functional groups and the chemical affiliation are analyzed by FTIR spectra. The potential of CuO Nps for the synthesis of nano-drug studied employing Zeta potential. SEM images show and confirm the morphology of the prepared Nps. The UV-DRS was used to monitor the bandgap energy of CuO Nps. The prepared samples have inhibiting higher to *Flavobacterium psychrophilum* bacteria. Hence, the current approach could be a facile, cost-efficient, reproducible, eco-friendly, and green methodology. Our report adscititious the worth for the appliance of Nps in medical specialty and nanotechnology applications with the absence of adverse facet effects.

Keywords: Antibacterial Activity, *Morinda citrifolia* Leaf, SEM, UV-DRS, Zeta Potential.

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INTRODUCTION

The synthesis of metal oxide Nps by the green method is an emerging research area. As the metal particles take bulk characterizes of the Nps disappears to be alternatively to that of a quantum dots¹⁻³. Nanotechnology has created new opportunities for advances in many medical and biological applications such as cancer treatment, tissue regeneration, biosensing, medical imaging, and diagnosis, etc due to their novel properties that differ from their bulk materials^{4,5}. Nanotechnology researches are the priority field and the forefront of modern research in recent years in different sciences such as medicine, physics, chemistry, biology, etc. Nanomaterials with their distinctive physical and chemical properties supported by numerous applications by dynamic their size, distribution, and morphology. Multiple methodologies were developed during this field to synthesize biocompatible Nps to fulfill the precise desires, in the main within the applications associated with the medicinal field. Metal Nps have recently attracted important attraction because of their explicit morphologies which are account for various applications⁶. The distinctive properties of metal oxide Nps arise from particularly their high surface-to-volume quantitative relation, the applications of metal oxide Nps, are receiving prime interest from researchers for their applications. CuO Nps are synthesized employing numerous conventional approaches; for instance: co-precipitation, sol-gel method, high energy ball milling, electrochemical and electrophoretic depositions, chemical vapor deposition, chemical vapor, and microwave-assisted combustion methods Noticeably, physical and chemical approaches may require high energy and also produce toxic byproducts

affecting the humans and the environment, while the biological approach is a single step for NPs synthesis with non-utilization of toxic chemicals, inexpensive equipment, and eco-friendly. The metal Nps differs from that of the majority material and can consequently be easily taken in. Among all Nps, copper oxide Nps (CuO Nps) have engaged notable dedication representing, copper is help to the development of the modern technologies.^{7,8} The world has been using Cu complexes for different purposes for a century, such as an algacide, fungicide, water purifier, and antibacterial. Copper-based compounds are efficient biocidal properties, which are generally used in several health-related applications. In recent years, CuO Nps have increased great interest and consideration for the reason of their physicochemical properties; it has been focused on CuO Nps due to their optical, and mechanical properties^{9,10}. In a different preparation method, the green technique of preparing metal oxide Nps is very good due to its low cost, economical, unique reproducible. Synthesis of Nps employing plants can likely render more biocompatibility to the Nps. The preparation using plants tends to be very faster than using micro-organism and is comparable to very easy for the production of Nps¹¹. Using plants for the biogenesis of Nps may be a significantly helpful approach than different biological ways in which because it eliminates the challenges related to microbial culture maintenance. The herbal plant extract has been used for the synthesis of Nps. Plant extract contains alkaloids, quercetin, polysaccharides, and the quantity of an optional metabolite which assume a basic part during the Nps union by going about as lessening agents¹². *Morinda citrifolia* plant is used as a traditional Ayurveda medicine. *Morinda citrifolia*, popularly called noni, could be a plant of significant high worth in many fields of applications. The employment of its structures is dated by the Polynesians for quite 2000 years, notably because of its therapeutic properties associates with skin diseases, tuberculosis, burns, and arthritis, among others. These bioactive compounds like minerals, fatty acids, vitamins, amino acids, and saccharides are promising for therapeutic applications, though the medical and nutritional values of this plant. These articles aimed to prepared nano-sized CuO in an effective technique and analysis the crystallite nature, composition, and interaction of Nps with the reducing agent. This paper was discussed by the formations, stability, crystalline size of CuO Nps in aqueous solutions are confirmed using XRD, FTIR, UV-DRS, and SEM analysis. Also, Zeta Potential and Antibacterial studies were carried out for the sample.

EXPERIMENTAL

Material and Methods

Copper sulphate was purchased from Madras Scientific Suppliers, India. AR grade chemical was utilized in the experiments. The deionized water was used all over the experimentation for making solution and washing purposes.

Plant Material Collection and Preparation of Aqueous Leaf Extract

The leaves of *Morinda citrifolia* belonging to the family Rubiaceae were collected from the garden in Tirunelveli District. The collected leaves of *Morinda citrifolia* leaf were cleaned with running tap water to remove adhered dust impurities and other contaminants, followed by double-distilled de-ionized water. They were grinded with the help of a mixer grinder and then the extract was taken from the grinded paste. It was then filtered and used for the preparation process.

Preparation of Reduced CuO Nps

A standard solution of 0.2 M copper sulphate ($\text{CuSO}_{4.5}\text{H}_2\text{O}$) was prepared and the leaves extracts were added to the standard solutions of $\text{CuSO}_{4.5}\text{H}_2\text{O}$ until precipitate forms. The mixture was then stirred for about four hours. A color change was observed from blue color to a pale green-colored solution. The initial formation of CuO Nps was determined by its color change shown in (Fig.-1) the complete process takes place at room temperature. The observed color change indicates the reduction of copper ions and the formation of CuO Nps with a pH value of 7. The precipitate is collected and dried in an oven for one day at 100°C followed by a calcinations process at the temperature of 500° C for four hours with the resulting yield of about 2.75 g, respectively. The dried precipitates were made into a fine powder with the help of a mortar and were used for further characterizations.

Detection Method

The structural characteristics of synthesized Copper Oxide Nps using leaves extract of *Morinda citrifolia* were determined through the X-Ray Diffractometer X'PERT PRO diffractometer 34 radiation ($\lambda=1.5406 \text{ \AA}$) available at the School of Physics, with Cu-K Alagappa University, Karaikudi, Tamil Nadu, India. and the functional groups from the extract were explored from Fourier Transform Infrared spectroscopy. UV-DRS was analyzed using UV - Visible Spectrophotometer(model SHIMADZU/UV 2600), Heber Analytical Instrumentation Facility at Bishop Heber College Tiruchirappalli. The morphology was analyzed by SEM (SEM; JEOL – model JFC1600), Karunya University, Coimbatore. The Zeta Potential was recorded by Malvern Zetasizer Nanosizer, Karunya University, Coimbatore.

Fig.-1: Reaction Process of Formations of CuO Nps

RESULTS AND DISCUSSION

XRD Studies

The sample of CuO Nps could be characterized using XRD analysis to confirm the nature of CuO Nps. XRD pattern of the synthesized CuO is depicted in Fig.-2 with its reference peak. The peak intensities give peak position and also details about the electron density inside the unit cell. The major peaks obtained for Cu ONps, corresponding hkl values, and the XRD parameters found from XRD analysis is (111), (0 2 0), (2 1 2), (0 3 2), (1 3 3), (2 3 2), (1 4 1). The obtained values are well coincidentally with the JCPDS file No: 7718 98 confirms the formation of an amorphous orthorhombic structure. The average crystalline size of the CuO Nps was determined as 28.2 nm. The Bragg's peaks indicate the reduction in crystalline size.^{13,14} The crystalline diameter of CuO Nps was found from the FWHM of the XRD using Scherrer's formula:¹⁵

$$D_{hkl} = k \lambda / \beta \cos \theta$$

Where, D is the average crystal domain size and β is FWHM.

Fig.-2: XRD Pattern of CuO Nps Sample

FTIR Spectral Analysis

FTIR vibrational spectroscopic studies were used to identify the existence of various functional groups in biomolecules such as alkaloids, carbohydrates, flavonoids, proteins in the plant extract. In Fig.-3, the intense broadband at 3431 cm^{-1} corresponds to the stretching vibrations of OH group indicating the presence of water. The band at 1626 cm^{-1} corresponds to C=O stretching (aldehydes and ketones). The peak at 669 cm^{-1} and 2922 cm^{-1} attributed to the stretching vibration of the C=H and H-C-H group that is characteristic peaks after the synthesis of CuO Nps^{16,17}. The peak at 465 cm^{-1} is assigned to the confirmation of CuO Nps. These results indicate that Nps is capped with the chemical compound present within the leaf extracts.

Fig.-3: FTIR spectra of CuO Nps

UV- DRS Analysis

The UV-DRS characterizations were performed to analyze the electronic properties of the prepared CuO Nps as shown in Fig. is synthesized by using *Morinda citrifolia* leaf extracts. The samples have strongly measured radiation peak below at 570 nm. The E_g of CuO was found from the spectrum is 2.17eV. The reflectance spectrum is also found using the Kubelka-Munk relations. To convert the reflectance data into a Kubelka-Munk functions $F(R)^7$, the subsequent relation was used by $F(R) = (1-R)^2/2R$. Figure-4 shows tauc's plot for direct electronic transitions of CuO Nps direct bandgap (2.17eV) is higher as compared to bulk values; this blue shift in the direct bandgap is due to the quantum confinement effect.¹⁸ Optical absorption shows that the direct bandgap is higher the materials will be crystalline in nature.^{19,20} The E_g was increasing with the reductions of its size due to the quantum confinement effect.

Fig.-4: UV-DRS spectra of CuO Nps.

SEM Analysis

The SEM analysis has provided the size and morphology details of prepared Nps. The SEM images reveal that the samples consist of an outsized range of dispersive Nps within 28.2 nm. Since particle size will increase the grain size linearly, agglomerations were reduced with a rise in grains growth. The agglomeration of a particle is sometimes explained as a typical way to minimize their surface free energy²¹, and therefore the SEM images for CuO Nps are shown in Fig.-4. SEM clearly shows the surface choices, by that it highlights that CuO Nps was synthesized.

Fig.-5: SEM images of CuO Nps.

Antibacterial Activity of CuO Nps Sample

One expected relationship between Nps and the antibacterial proceedings is as follows: “Nanomaterials, as antibacterial enhances to antibiotics, are utterly alert and are gaining huge interest as they are going to fill the gaps where antibiotics frequently fail”. To boot, nanomaterials can complement and support ancient antibiotics “as an honest carrier.”^{22,23} The prepared CuO were analyzed for antimicrobial activity by the agar well diffusion methodology²⁴ against completely different sorts of pathogenic bacteria *enterobacter sp*, *Escherichia coli*, *Flavobacterium psychrophilum*, and *Klebsiella sp*, during this methodology Nutrient, agar media plates were seeded with 18 to 24-hour cultures of microbial inoculums. Whatman No. one filter paper disc (6 millimeters in diameter) was placed with the assistance of a sterile extractor on the media and then nano sample extracts in a volume of 6 μ l (1mg discs) were applied on the disc. The media plate was incubated at 37°C for 18 to 24 hours in an incubator. The chemical diffused from the disc into the agar media thus preventing the growths of microorganisms (if susceptible) in the area around the discs known as the zone of inhibition. Following inhibition, different levels of the zone of inhibition formed around the well were measured. The level of inhibition against the above bacteria is depicted in Fig.-5. CuO-Nps discharged 253 $\times$ a lot of ions than Ag Nps, manufacturing higher antibacterial activity, presumably because of CuO higher oxidation susceptibility.²⁴⁻²⁷ it was ascertained that CuO Nps victimization *Morinda citrifolia* leaf inhibiting higher to *Flavobacterium psychrophilum* bacteria compared to other bacterias and hence this sample can be used in fabricating medicines such as mucus, brain, mucus, spleen. The better uses of Nps in medicine have led to an evolvment number of studies curious about the potential anti-bacterial mechanism of Nps.

Zeta Potential Analysis

Nps not solely will combat bacterial and microbic resistance themselves it may also act as a “medium and carrier” of antibiotic. Zeta-potential, tell us the character of a charge on and related to particles, additionally the distribution of charge hooked up to the particle and within the encompassing solvent. Zeta potential studies are important property which can play important roles in the effectiveness of nanomedicine. Zeta potential is widely employed in pharmaceuticals for stability evaluation. It was mayhap positive or negative. The zeta potential for the synthesized CuO Nps is - 6.45mv is shown in Fig 6. Nps surface may be a vital thought in focus drug deliveries.

In fact, among the blood, typical Nps and charged particles are going to be quickly cleared by built macrophages. The difference of the particles surface charges might probably control, binding to the direct Nps to cellular compartment each in vivo and vitro.^{28,29} The cellular surface is influenced by charged

sulfated proteoglycans molecule that plays a pivotal role in cellular motility, migration, and proliferation it as confirmed from Fig.-5.³⁰ It's conjointly investigating the domination of CuO Nps on bacterial gentrification by inflicting major alterations of the expressions of a key protein. Once getting into the cell, proteomics bioinformatics studies show that CuO Nps caused regulation of protein concerned in chemical element metabolism and substance transport.

Fig.-6: Antibacterial Activity of the CuO Nps.

Fig.-7: Zeta Potential of CuO Nps.

CONCLUSION

The green preparation of CuO Nps using *Morinda citrifolia* leaves extract was found to be eco-friendly. The presences of a phytochemical constituent in the leaf extracts help in the preparation of the CuO Nps by inducing a redox reaction. Studies show that biomolecules like protein and flavonoids not only play a role in reducing the ion to the nanosize The functional groups of phytochemicals like alkane, amine, and hydroxyl groups team evoked the formations of Nps. The confirmation of the CuO was obtained using the UV-DRS at 570 nm. X-ray diffraction spectroscopy analysis determines the crystalline nature of

the particles and the quality of compounds. The particle size of CuO Nps using XRD is 28.2 nm. The FT-IR absorption peak observed at 465 cm⁻¹ in the IR spectra of synthesized CuO Nps corresponds to Cu-O stretching in the orthorhombic phase of CuO. The SEM image confirms the presence of crystalline CuO Nps of nearly similar shapes. Zeta potential popularly remains as an accepted predictor of bacterial surface charge. Also, it may be new useful methodology exploitations, affordable precursors, for the preparation of metal oxide Nps. Our team's future analysis is progressing to be specializing in formulating the eco-friendly prepared CuO Nps in fabricating medicines. Thus CuO Nps was one of the important properties which could play an important role in the effectiveness of nanomedicine.

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[RJC-6252/2020]