Biogenic synthesis of copper oxide nanoparticles using leaf extracts of *Cissus quadrangularis* and *Piper betle* and its antibacterial effects

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Owing to its environment-friendly, biocompatible, non-toxic nature of nanoparticles attained from green plant extract, it has remarkable significance in the field of nanotechnology towards effective biomedical application. This work reports the synthesis of copper oxide nanoparticles by means of *Cissus quadrangularis* and *Piper betle* via green-synthesis route. The medicinal leaf extracts possessing phytochemicals such as alkaloids and polyphenols act as a reducing agent in the current synthesis. The characterization of functional groups of CuO NPs by two different leaf extracts was investigated by FTIR analysis which measures reduction and stabilization of CuO NPs. X-ray diffraction confirms the orthorhombic structure with an average crystallite size of about 32.54 and 32.09 nm. The irregular spherical-shaped morphology of the samples is evidenced from scanning electron microscopy analysis. The transmission electron microscopy images demonstrated 50 nm sized prepared CuO nanoparticles. The bioactivity of the prepared sample is confirmed from antibacterial studies and zeta potential.

1 | INTRODUCTION

Nanotechnology has shown explosive growth worldwide in the past few years in the development of engineered nanomaterials with a wide range of applications in various fields ranging from material sciences to biomedicine [1]. The novel properties of nanoparticles (NPs) have been exploited in a wide range of possible applications such as medicines, renewable energies, environmental remediation, and biomedical devices. Nanobiotechnology is an interdisciplinary field that has emerged as an interaction between biotechnology and nanotechnology for developing new biosynthetic devices and eco-friendly technology with advantages in the field of drug delivery to bio-chips [2, 3]. Thanks to the controlled size and composition, metal oxide NPs gains provide fundamental and technological attention as they offer solutions to technological and environmental challenges [4–7]. The green synthesis of NPs employs plant material to meet the growing need in developing environmentally friendly approaches [8–11]. Medicinal plants have been playing a crucial role in maintaining human health and civilizing the quality of human life for thousands of years. Ayurveda, unani, homeopathy, naturopathy, siddha, and other alternative medicinal systems have been utilizing plants as effective medicines to cure various diseases [12–14]. Plants are valuable source of compounds with reductive abilities called antioxidants. The higher bioactivity of as-synthesized
nanoparticles resulted from their large surface area and preferential adsorption of the bioactive constituents from the extract onto the surface of the nanoparticles, both of which are strongly affected by the surface area, particle size and surface reactivity of the nanoparticles [15, 16].

Green production of NPs involves the reduction or elimination of toxic reagents usage, which ends in a significant reduction in the number of harmful residues to human health and the environment [17–20]. The significance of this synthesis technique results from its efficiency in the choice of green alternatives for solvents, reducing agents, catalysts, and other stabilizing components involved in chemical reactions to protect the environment from pollution. The green-based methods are being developed to replace chemical methods to improve quality, to be eco-friendly, and to avoid toxicity. The advantages of the green synthesis process for the production of copper oxide (CuO) NPs using plants are rapid, eco-friendly non-pathogenic, and economical protocol [21, 22]. Moreover, it provides a single-step technique for the plant-mediated synthesis of nanoparticles resulting from their simplistic preparation and several other purification stages [23]. In particular, CuO NPs have attracted increasing interest due to their sole properties and varied applications in various fields. The extracts from plants are composed of widespread biomolecules such as alkaloids, proteins, vitamins, tannins, enzymes, polysaccharides, quercetin, which facilitates reduction, capping, and stabilization of CuO NPs [24, 25]. Cu NPs exhibit strong antimicrobial activity. Metallic nanoparticles play a crucial role in inhibiting the growth of bacteria in solid and aqueous media due to their large surface-to-volume ratio. Metallic nanoparticles and nano-sized carriers for drug delivery prove their effectiveness in treating infectious diseases in vitro as well as in animal models. Copper oxide nanoparticles are potentially effective against various bacterial pathogens concerned with hospital-related infections. They can be another toll for an antibiotic, antifungal, and antimicrobial agent in the plastics, coatings, and textiles industry. In the future, the main advantage is the fabrication of copper–copper oxide-capped nano-fibrillar cellulose: an efficient new biomaterial. Copper is replaced with other noble metallic entities due to its high conductivity and low cost. The synthesized CuO NPs using green synthesis have potential use in different applications such as ecological areas, catalytic properties, biomedical field, antimicrobial activities, and drug delivery systems [26, 27]. In recent years, biomaterials have been widely used as a means of green technology for the synthesis of spinel ferrites [28, 29].

The synthesis of CuO NPs using plant constituents has not yet been studied for a large number of natural compounds. The present work is an attempt to use *Cissus quadrangularis* plant leaf extract and *Piper betle* leaf extract as a bioreductant for green and rapid synthesis of CuO NPs by using copper sulphate as the parent material. The formation of CuO NPs was primarily confirmed by the colour change of the attained solution. In this study, the synthesis of CuO nanoparticles has been investigated by X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transformation infrared spectroscopy (FTIR), and zeta potential (ZP) studies. The structure, morphology, chemical bonding, and antibacterial nature are reported.

2 | MATERIALS AND METHODS

2.1 | Materials

Analytical grade copper sulphate used for the synthesis of CuO NPs as the copper precursor was purchased from Hi-Media, Tamil Nadu, India, and deionized H₂O was utilized for the extraction and synthesis. The leaf of *C. quadrangularis* and *P. betle* used in this work was collected from the nearest garden located in Tirunelveli District, Tamil Nadu, India.

2.2 | Synthesis of copper oxide nanoparticles

CuO nanoparticles were synthesized by the co-precipitation method. The leaves of *C. quadrangularis* and *P. betle* were collected and washed thoroughly with distilled water to make them free from dust particles and surface contamination. They were grinded with the help of a mixer grinder and the extract was taken from the grinded paste. It was subsequently filtered and used in the synthesis process. Copper sulphate (CuSO₄·5H₂O) solution of 0.2 M was prepared using double distilled water. The prepared leaf extract of *C. quadrangularis* and *P. betle* was added dropwise into the CuSO₄·5H₂O solution separately until the precipitate forms; the complete process takes place at room temperature. The observed colour change indicates the reduction of copper ions and the formation of copper oxide nanoparticles with a pH value of 7 as shown in Figure 1. The precipitate is collected in a petri dish and dried in a hot air oven for 1 day at 100°C followed by a calcination process at the temperature of 500°C for 4 h with the resulting yield of about 2.97 and 3.39 g, respectively. The dried precipitates were made into a fine powder with the help of a mortar and were used for further characterizations.

2.3 | Physical measurements

The structural characteristic of synthesized CuO nanoparticles using leaves extract of *C. quadrangularis* (CS1) and *P. betle* (CS2)
RESULTS AND DISCUSSION

Copper oxide NPs formed are necessarily subjected to XRD analysis for the measurement of the size of these particles. Figure 2 shows the XRD pattern obtained for the CuO NPs synthesized using the leaf extract of C. quadrangularis (CS1) and P. betle (CS2). XRD results reveal the crystalline nature and size of CuO nanoparticles. The distinctive peaks located at 2θ values is in good agreement with those of powder CuO obtained from the JCPDS card no. 78-1588 (CS1) and 77-1898 (CS2) confirming the formation of an amorphous orthorhombic structure.

The distinctive peaks located at $2\theta = 18.78^\circ$, $24.09^\circ$, $29.65^\circ$, $32.71^\circ$, $33.08^\circ$ and $36.37^\circ$ are assigned to $(1 0 1)$, $(0 1 2)$, $(0 2 0)$, $(2 0 0)$, $(1 0 3)$ and $(1 1 3)$ plane orientation of CuO NPs. The distinctive peaks observed at $2\theta$ values is 13.47°, 16.15°, 22.37°, 27.56°, 30.19°, 33.13°, 35.43° and 37.23° are assigned to $(1 1 1)$, $(0 2 0)$, $(2 1 2)$, $(0 3 2)$, $(1 1 5)$, $(2 3 2)$, $(1 4 1)$ and $(0 2 6)$ plane orientation of CuO NPs. The average crystalline size of CuO NPs synthesized using the leaf extract of C. quadrangularis (CS1) and P. betle (CS2) was found to be about 32.54 and 32.09 nm using the following Debye Scherrer formula [29–32].

$$D = \frac{k\lambda}{\beta \cos \theta}$$

where $D_{hkl}$ is the grain size, $k$ is the dimensionless shape factor (0.94), $\lambda$ is the wavelength of X-ray, $\beta$ represents the line broadening at full width at half maximum (FWHM), $\theta$ is the Bragg's angle.

FTIR analysis reveals the involvement of bioactive molecules present within the leaf extract of C. quadrangularis and P. betle that act as redox precursors for the formation of copper oxide nanoparticles as depicted in Figures 3 and 4. The absorption peaks shown at 3385 cm$^{-1}$, 3317 cm$^{-1}$ correspond to N–H/O–H stretching and the peaks at 2923 cm$^{-1}$, 2899 cm$^{-1}$ correlate to the C–H stretching of amides and alkanes. The presence of a band at 2361 cm$^{-1}$, 2332 cm$^{-1}$ is attributed to alkynes. The band at 1626 cm$^{-1}$, 1622 cm$^{-1}$ represents the C–O stretching of the COOH group. The existence of band at 536 and 612 cm$^{-1}$ are recognized to be the vibrations of Cu–O, confirming the formation of copper oxide nanoparticles [33, 34]. The above-mentioned results confirm the existence of phytochemicals such as alkaloids, flavonoids, tannins, proteins, and carboxylic acids which were responsible for the bioreduction and stabilization of copper oxide nanoparticles.

The insight information about the morphology of biosynthesized CuO NPs the CS1 and CS2 using leaf extract of C. quadrangularis and P. betle were examined by SEM and TEM. A clear indication of the morphology of CuO NPs can be seen from SEM and TEM images portrayed in Figure 5.

The distinctive images demonstrated that the particles were spherical with some surface agglomeration with a controlled grain size of the particles complemented by the leaf extract of C. quadrangularis and P. betle. The nature of CuO NPs (CS1 and CS2) was reported by TEM analysis which is in agglomerated cluster structure as depicted in Figure 5e,f. The obtained CuO NPs are evidenced with an average size of 50 nm. The agglomeration was reduced significantly with the increase in grain growth [35, 36]. On increasing the concentration of plant extract into the parent solution, the particle size is increasing with increasing quercetin present in the leaf extract. Since quercetin is a pure biomolecule, the minimal concentration was sufficient to reduce
particles to improve their biomedical applications [43–45]. Thus, a stronger charge can increase the stability of the particles with positive or negative zeta potential tend to aggregate more with the increase in grain growth [25].

Zeta potential analysis was carried out to detect the surface charge and reliability of biosynthesized CuO NPs as nanocarriers. The potential stability of NPs with the average zeta potential value of −5.26 and 0.154 mV for biosynthesized CuO NPs using C. quadrangularis and P. betle leaf extract are respectively shown in Figure 6. The mechanism could be explained through decreased potential giving rise to an increase in attractive forces to overcome the repulsion, thereby causing the flocculation and breaking up of dispersion. Previous literature suggested that the charged particles are often rapidly opsonized and massively cleared by fixed macrophages [37,38]. The NPs with each negative zeta-potential led to massive staining of retinal cells after intravenous infusion. The scale limitation for penetration of the nano-carriers into the extracellular space was proposed to be within the order of 64 nm [39–41] or maybe 20 nm which is the specific size of the pore for the extracellular space as manifested by microscopy [42]. The charged nanoparticles with positive or negative zeta potential tend to aggregate more rapidly. A stronger charge can increase the stability of the particles to improve their biomedical applications [43–45]. Thus, the present work demonstrated the obtained particle size, shape, and surface charge of CS1 and CS2 samples that evidenced their significance in targeting drug delivery. The rich source within the leaf extract maybe liable for the reduction of metal ions and for the efficient stabilization of Bio-CuO NPs [46].

The use of CuO NPs, as new agents for inhibition of microbial growth, has emerged due to the development of antibiotic resistance. The antibacterial activity of the synthesized nanoparticles was studied. Antibacterial activities of the synthesized copper nanoparticles were tested against human pathogenic bacteria, namely, Enterobacter sp., Escherichia coli, Flavobacterium psychrophilum, and Klebsiella sp. bacteria by disc diffusion method [47–50]. The 50 μl of the test sample with a concentration of CuO NPs of 11.2 μg ml⁻¹ was used against each strain swabbed on nutrient agar plates followed by incubation at 37°C for 24 h.

The levels of inhibition against the above-mentioned bacteria and their bar diagram are depicted in Figure 7. It was observed that in CuO NPs exploitation C. quadrangularis leaf inhibits higher results to Enterobacter sp. bacterium compared to a different bacterium that showed the potentiality of this sample in fabricating medicines for medical specialty illness [51–54]. Moreover, CuO NPs using P. betle leaf inhibit a higher effect on Klebsiella sp. bacteria compared to other bacteria confirming their ability to be used as antibiotics in K. pneumonia infections. The final results of antibacterial action studies suggest that the biosynthesized CuO NPs have greater antibacterial activity even at lower doses [55,56]. Thus, the bio-CuO NPs get connected very easily to the cell membrane, and its further penetration inside the bacteria is also evidenced by ZP studies.

4 | CONCLUSION

The biosynthesis of CuO NPs was successfully reported using C. quadrangularis and P. betle leaf extract by the co-precipitation method. The sharp narrow peaks from the X-ray diffraction pattern indicate the crystalline structure with the orthorhombic phase of CuO NPs. The SEM and TEM images confirmed the spherical morphology of the as-synthesized NPs. The particle size of CuO NPs analysed by XRD is corroborated by SEM micrographs. The presence of phytochemical constituents in the leaf extract induced as a reducing agent for the formation of CuO NPs is explored by FTIR. The antibacterial activity of CuO NPs is authenticated by the reliability of zeta potential.
Based on the above results, the biosynthesized CuO-NPs have an effective antibacterial activity which could be because of the entrance of the NPs to the cell membranes resulting in the termination of the cell divisions. This highlights that there is interactivity between the bacteria’s cell wall and the metal oxide nanoparticle is verified by the reliability of zeta potential. Thus, our work suggests that CuO NPs synthesized from herbal leaf extracts could have great potential in the fabrication of nano-drugs.

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