Research Article

Anti-inflammatory and Antimicrobial Potential of Cissus quadrangularis-Assisted Copper Oxide Nanoparticles

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Recently, nontoxic origin-mediated synthesis of copper oxide nanoparticles acquires further recognition because of the key role of bioapplications. The plant Cissus quadrangularis is one of the prominent herbs used in the treatment of diabetes, asthma, tissue regeneration, etc. In this study, we tested the process of copper oxide nanoparticle synthesis and their role in many functions from Cissus quadrangularis. The synthesis of copper oxide nanoparticles uses plant extract and characterization by X-ray diffraction, thermogravimetric analysis (TGA), Fourier-transform infrared spectroscopy (FT-IR), atomic force microscope (AFM), and scanning electron microscope (SEM). The synthesized nanoparticles were analyzed for their biomedical applications such as antibacterial, antifungal, antioxidant, antidiabetic, and anti-inflammatory activity and antiproteinase action. The results show that the C. quadrangularis plant-mediated nanoparticles may be used in many biomedical applications related to arthritis, diabetes, and the production of various antimicrobial products in the future.

1. Introduction

The biosynthesis of nanoparticles is the collaboration of both fields of biotechnology and nanotechnology which involves biomaterials commonly used for the synthesis due to various advantages, but most importantly is due to its environment-friendly nature [1]. The importance of green nanotechnology has become a latest trend among the researchers lately, as it does not require any utilization of hazardous chemical or reagents; these reducing or stabilizing agents are nontoxic, biocompatible to the environment as well as for any biomedical applications. These agents include microorganisms, parts of plants, enzymes, or green chemicals like chitosan [2], curcumin [3], and chitin [4]. Copper nanoparticles are slightly toxic in nature. To curb in toxicity sulfidation of CuNP can be performed to form copper oxide nanoparticles [5]. Copper sulfide is an interesting area of research because of its ability to mould into various morphologies; it is a member of transition metal chalcogenides, and behaves like a p-type semiconductor where copper vacancies act as acceptor sites like digenite (Cu1.8S), sulphur rich covellite (CuS), copper rich chalcocite (Cu2S), anilite (Cu1.75S), or djurleite (Cu1.95S). The ability to form nonstoichiometric phases is because of the variable valence states of copper which has a formula of CuXSy [6, 7]. Various methods have been employed for the synthesis of these nanoparticles like laser induction [8], pulse laser ablation [9], solvothermal [10], phase-controlled synthesis [11], sonochemical [12], or electrosynthesis [13]. The major setback of these mentioned methods is the requirement of...
complicated or expensive equipment and external usage of stabilization agents along with reducing agents. This can result in irregular impact in our surroundings; therefore, the utilization of phytochemicals from plants can be better alternative to this issue, which can act as both reducing and capping agent [14].

The antimicrobial evaluation is important to be considered as it has become a major threat to human via contaminated water or food. The demand for disinfectants has increased tremendously, then protection against food contamination which can be done by packaging, or the development of antibiotics that can be used against pathogenic agents or new formulation against multidrug-resistant (MDR) pathogens [15]. Oxidative stress is caused due to high generation of ROS, which may lead to chronic inflammation; therefore, production of anti-inflammatory agents that can act as antioxidant agents which reduces the oxidative stress caused is also mandatory now [16].

This paper suggests the exploitation of copper oxide nanoparticles using fresh leaves of *Cissus quadrangularis* through various biomedical applications like antimicrobial, antioxidant, and anti-inflammatory or analyzing the anti-proteinase activity.

2. Materials and Methods

For the synthesis of nanoparticles, the fresh leaves of *Cissus quadrangularis* were collected from Vellore district. The doubled-distilled water was used for the entire experiments. All the reaction was completed with glassware washed and rinsed by distilled water and dried in hot air oven [17]. The filtration was done by Whatman No.1 filter papers.

2.1. Plant Details. *Cissus quadrangularis* in South Indian treebine is a climbing, foetid shrub. The outermost layer of the stem is blackish to reddish; division of stem is densely pubescent, swollen at node (hold one or more leaves), and 4 petals are present [18]. It acts as a drug for local tissue injury that is better remedy and relief from wound problems and swelling issues.

Herbal plants have medicinally important components in their various kinds of parts like stem and leaves [19]. The synthesis of nanoparticles by plants depends upon the nature of plant that is their phytochemical compounds, specific adaptation, and medicinal values. In this study, we report the eco-friendly and cost-effective green synthesis of copper oxide nanoparticle using leaf extract of *Cissus quadrangularis*.

Botanical name: *Cissus quadrangularis*

Family: VITACEAE (grape family)

2.2. Preparation of Plant Extract. The fresh *Cissus quadrangularis* leaves were collected and thoroughly washed several times using normal water. Then, it was allowed to dry

### Table 1: Phytochemicals screening of the plant extract *Cissus quadrangularis*.

| S/No. | Phytochemicals       | Results          |
|-------|----------------------|------------------|
| 1     | Carbohydrates        | Reddish colour   |
| 2     | Tannins              | Greenish blue    |
| 3     | Flavonoids           | Yellow           |
| 4     | Alkaloids            | Greenish         |
| 5     | Anthraquinone        | Pink             |
| 6     | Anthacyanaside       | Negative         |
| 7     | Steroids             | Red colour layer |
| 8     | Terpenoids           | Negative         |
| 9     | Saponins             | Foam layer       |

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2.3. Phytochemical Screening of Plant Extract. Medicinal plants are the richest biosource of drugs of traditional system, pharmaceuticals, neutraceuticals and food source, and plants are the richest biosource of drugs of traditional systems. Extraction is the separation of medicinal active parts and tissues of plants with using of solvents by standard procedure [17]. The plant extracts and methanolic, ethanolic solvents were assessed for the phytochemical screening of carbohydrates, tannins saponins, flavonoid, alkaloids, anthraquinone, anthocyanide, steroids, and terpenoids by using standard methods.

2.4. Synthesis of Plant-Mediated Nanoparticles. The fine dried powder of *Cissus quadrangularis* extract were used for the synthesis. 1 g of plant powder was dissolved in 100 mL of double-distilled water and kept for boiled in oven 2 minutes. This mixture was filtered by Whatman filter paper. The 20 mL of filtered solution was mixed with 80 mL of distilled water [20]. Then, 10 mM of copper sulphate was added to this mixture and kept in shaker for 24 hours. After incubation, the solution was centrifuged for 10 minutes at 10000 rpm. Then, the pellet was collected and dried by hot air oven as shown in Figure 1 [17].
2.5. Characterization. Characterization is essential to determine the average size, shape, and features of the synthesised nanoparticles. These bioactive nanomaterials are analysed by UV-Vis spectrophotometer, X-ray diffraction (Bruker–D8 advance, Germany model), thermogravimetric analysis (SDT Q600 V20.9 build 20), Fourier-transform infrared spectroscopy (FT-IR), atomic force microscope (AFM), and scanning electron microscope (SEM) [21].

**Figure 8:** AFM data for *C. quadrangularis*-mediated copper oxide NPs.

**Figure 9:** SEM and EDAX data for *C. quadrangularis*-mediated copper oxide NPs.

| Clinical isolates         | Ab | Ab+NP | NP |
|---------------------------|----|-------|----|
| *Staphylococcus aureus*   | 16 | 21    | 11 |
| *Streptococcus sp.*       | 15 | 18    | 26 |
| *Seratia marscenes*       | 8  | 10    | 20 |
| *Escherichia coli*        | 25 | 26    | R  |

**Table 3:** Antibacterial activity against plant-mediated synthesis CuO NPs.
2.6. Applications

2.6.1. Antibacterial-Enhanced Method. Antimicrobial activity of copper oxide-mediated synthesis of nanoparticle acts against pathogenic bacterial strains (Staphylococcus aureus, Streptococcus, E. coli, and Serratia marcescens [17]). To determine the zone of inhibition was observed using MHA agar [22]. Muller Hinton agar was prepared and sterilized at 120 lbs for 45 minutes. Media were poured on the respective plates; after solidification, the disc was placed and kept for incubation at 37°C for 24 hours. After incubation, the zone of inhibition was measured [23].

2.6.2. Antifungal Activity. Antifungal activity of biosynthesised copper oxide nanoparticle was determined against four fungal strains (Candida albicans, Aspergillus niger, Aspergillus flavus, Aspergillus aculeatus). This action was done by disc diffusion method using potato dextrose agar [24]. Pour the sterilized media on the plates allowed to solidification. The disc was placed on the plates and kept for incubation at 25°C for 48 hours after treated with UV radiation [25].

2.6.3. Antioxidant Activity. Antioxidant activity was performed by DPPH (1,1diphenyl-2 picryl hydrazyl) assay to CuO nanoparticle [26]. Antioxidants play a major role in health protecting factor; it is reducing the chronic disorders [27]. This method gives the antioxidants possible potential of quadrangularis-mediated synthesis of NPs. Free radical scavenging activity of different concentration (50 μg/mL, 100 μg/mL, 150 μg/mL, and 200 μg/mL) of nanoparticles by 0.01 Mm of DPPH (39.432 g/mol) were prepared dissolved in ethanol [27]. The prepared DPPH solution was incubated for 30 minutes at dark place. Then, solution (2 mL) was added to the different concentration NPs, and 2 mL of ethanol was added and incubated for 30 minutes. The reference standard (positive control) compound being used was ascorbic acid, the absorbance measured at 517 nm [28].

2.6.4. Antidiabetic Activity. Diabetes mellitus is a chronic disorder which leads to severe tissue damage and vascular damage due to insulin sufficient. The herbal products are playing major role in drug development [29]. Here, the Cissus quadrangularis-mediated synthesis of NPs was used to determine the efficiency against diabetes characterized by alpha amylase activity. To take the different concentrations of NPS (50 μg/mL, 100 μg/mL, 150 μg/mL, and 200 μg/mL), add the 500 μL of phosphate buffer containing alpha amylase (0.5 mg/mL) solution. The mixture was incubated for 10 minutes at RT. Then, 500 μL of 1% starch-containing phosphate buffer solution was incubated for 10 minutes. Then, add the 1 mL of DNSA (containing 0.5 g of dinitro salicylic acid, 15 g of sodium potassium tartrate diluted in 50 mL of distilled water). It was kept for 5 minutes in water bath [29]. The reaction mixtures make up into 5 mL of distilled water, and optical density was observed at 540 nm.

2.6.5. Anti-inflammatory Activity. Inflammation in biological response from damage cells (local tissue injury) is a difficult response that prevent the tissue damage and invasion of microbes through the small cuts, scratch, and abrasions [30].

Table 4: Antifungal activity against plant-mediated synthesis copper oxide NPs.

| Clinical isolates | Ab   | Ab+NP | NP  |
|-------------------|------|-------|-----|
| Candida albicans  | 7    | 22    | 17  |
| Aspergillus niger | 7    | 16    | R   |
| Aspergillus flavus| 7    | 8     | R   |
| Aspergillus aculeatus| 11| 14    | R   |

The membrane stabilization assay was performed by O positive blood. 10 mL of blood were collected with equal volume of Alsever solution (1 g of dextrose, 0.8 g of sodium citrate, 0.025 g citric acid, and 0.21 g sodium chloride in 50 mL) centrifuged at 3000 rpm, then the cells were separated and washed with isosaline (0.9 g of NaCl in 100 mL of distilled
water). Then, the different concentrations of NPs were mixed with HRBC (0.5 mL), phosphate buffer (0.5 mL), and 2 mL of hyphosaline (0.4 g NaCl in 100 mL). The mixture of solution was incubated at 37°C for 30 minutes and centrifuged at 3000 rpm. Then, the absorbance of haemoglobin content was estimated at 560 nm [31].

2.6.6. Antiproteinase Action. The herbal-mediated synthesis of nanoparticle was studied for anti-inflammatory activity by antiproteinase action. The reaction mixture contains 0.06 mg of trypsin, 1 mL of test Mm tris Hcl buffer, and different concentration (50 μg/mL, 100 μg/mL, 150 μg/mL, and 200 μg/mL) of nanoparticle. These mixtures of solution were incubated at 37°C for 5 minutes and centrifuged at 3000 rpm. Then, the absorbance of haemoglobin content was estimated at 560 nm [31].

minutes. The 2 mL of perchloric acid was added. The cloudy suspension were centrifuged, and the absorbance of nanoparticles were taken at 210 nm [32].

2.7. Statistical Analysis. All the data are represented as mean ± SD. The standard one-way ANOVA was used for standardization with p value < 0.05 which is considered as statistically significant.

3. Results and Discussion

3.1. Phytochemical Screening. The collected plant material was analysed by the phytochemical screening to check the presence of amino acid like carbohydrates and other compounds. Table 1 and Figure 2 shows the positive and negative result of the plant extract.

3.2. Characterization

3.2.1. UV–Visible Spectrophotometer. The characterization of nanoparticles begins with the UV-Visible absorbance, which depends upon the principle of SPR (surface plasmon resonance), where the conducting electrons get excited, and the absorbance is measured. The absorbance of nanomaterials observed ranges between the 300 and 700 nm. In this case, the copper ions reduced to nanoparticulate forms using the extract of Cissus quadrangularis. The reaction time taken was 4 h with maximum absorbance of 340 nm as shown in Figure 3 [22].

![Enhanced antifungal activity](image1.png)

**Table 5: C. quadrangularis-mediated synthesis of CuNo4 NPs.**

| Concentration | Standard | Control | NPs | Scavenging activity | IC 50   |
|---------------|----------|---------|-----|---------------------|--------|
| 50 μg/mL      | 0.084    | 0.934   | 0.245 | 73.76874            |        |
| 100 μg/mL     | 0.095    | 0.934   | 0.856 | 8.351178            | 264.69 |
| 150 μg/mL     | 0.102    | 0.934   | 0.867 | 7.173448            |        |
| 200 μg/mL     | 0.108    | 0.934   | 0.871 | 6.745182            |        |

![Figure 12: Antioxidant activity of copper oxide NPs.](image2.png)
3.2.2. X-Ray Diffraction Analysis. The size of the nanoparticles was calculated according to these peak values and crystalline size [33]. The synthesised herbal and fungal nanoparticles by copper sulfide size were found by the Debye-Scherrer equation from the $2\theta$ values and crystalline size from Table 2 and Figure 4.

$$D = \frac{k\lambda}{B \cos \theta} \text{ (Particle Size) = } \frac{(0.9 \times \lambda)}{(d \cos \theta)}$$

$$K = 0.9 \text{ (constant)}; \lambda = 1.54060 \text{ (constant)}; 2\theta = 29.72;$$

$$\theta = 12.86; B = 0.187 = 0.9 \times 1.5406 	imes 10^{-10} / 0.187$$

$$\times \cos 12.86 \times 0.01744$$

$$D = 42 \text{nm} B = \text{Full width half maximum (radian)} \quad (1)$$

3.2.3. TGA Analysis. From this result, to evaluate the loss of weight and peak of the temperature was observed [34, 35]. To find the weather, the nanoparticles increase or decrease their weight by TGA. The analysis of room temperature was observed from 800°C. The differential thermal analysis shows the (CuO-plant) 2.2060 mg ranges for synthesised materials. The thermal gravimetric analysis gave the weight loss of material at a particular temperature as shown in Figures 5 and 6.

3.2.4. Fourier-Transform Infrared Spectroscopy. The functional groups of Cissus quadrangularis plant-mediated synthesis of copper oxide nanoparticles is 3269.34 cm$^{-1}$ N-H stretch band due to presence of amine group. This primary amine produces two N-H absorptions, 2920.23 cm$^{-1}$ belongs to alkene group (C-H bond), and 1602.85 cm$^{-1}$ and 1438.90 cm$^{-1}$ have an aromatic compounds (C=C bond). From this analysis, it is suggested that antioxidant enzymes with amines, ethyl Ester, aromatic compounds, alkenes, and acetic acids might be absorbed on biosynthesized copper oxide nanoparticle as shown in Figure 7.

3.2.5. Atomic Force Microscope. Figure 8 shows the AFM images and express their surface size such as for scanned area range of C. quadrangularis-mediated synthesis of copper oxide NPs 70 to 100 nm. From this surface analysis, the particle size display the clusters of even line of profile [36].

3.2.6. Scanning Electron Microscope. It was observed 90 nm copper oxide nanoparticles synthesized using Cissus quadrangularis. The plant extracts exhibit different shapes of nanoparticles confirmed using SEM [37]. The presence of copper nanoparticle was confirmed by the EDX analysis, whereas the peaks gave the amount of particles in sample as shown in Figure 9.

| Concentration | I  | II | III | MEAN | STD DEV | STD ERR | Final       |
|---------------|----|----|-----|------|---------|----------|-------------|
| 50 μg/mL      | 1.286 | 1.334 | 1.378 | 1.33 | 0.046014 | 0.03 | 11.07 ± 0.21 |
| 100 μg/mL     | 2.055 | 2.145 | 2.223 | 2.14 | 0.084071 | 0.05 | 11.07 ± 0.22 |
| 150 μg/mL     | 2.257 | 1.278 | 1.345 | 1.63 | 0.546912 | 0.32 | 11.07 ± 0.23 |
| 200 μg/mL     | 3.157 | 3.234 | 3.279 | 3.22 | 0.061695 | 0.04 | 11.07 ± 0.24 |

**Table 6:** C. quadrangularis-mediated synthesis of copper oxide NPs.
3.2.7. Antibacterial Activity. The antibacterial activity of the copper oxide nanoparticle was observed by the antibacterial-enhanced method using control as streptomycin. Different formulations which involve the different mode of preparation or surface-decorated biomolecules of antimicrobial agents are produced which is cost effective, safe, and can also be treated against many MDR cultures [38]. The antibacterial activity was evaluated against *Staphylococcus aureus*, *Streptococcus*,
E. coli, and Serratia marcescens. The zone of inhibition measured between the three different modes such as in each space contains the antibiotic disc, antibiotic disc with nanoparticles, and nanoparticles. From the result, the nanoparticle copper oxide (26 mm) has a high effect against E. coli, the form of antibiotics with NPs as shown in Table 3 and Figure 10. The need to develop novel antibacterial agents has become an important task amongst researchers as a problem of resistance against bacterial cultures is being generated. The mechanism behind its antibacterial potential is explained by the interaction of the nanoparticles with outer surface of the cell membrane of the cultures, which eventually leads to disruption of the integrity of the membrane by producing pits in them, and increase of permeability causes a release of lipopolysaccharide molecules or other protein materials, and this results in cell death [39, 40].

3.2.10. Antidiabetic Activity. Alpha amylase activity of copper oxide NP shows the values about the inhibition of the concentration. Stabilization of membrane will produce the various disorder related to the inflammation due to release of lysosomal enzymes (as shown in Table 7 and Figure 14).

3.2.11. Anti-inflamatory Activity. Anti-inflammation activity is one of the assessments of anti-inflammatory activity. It played an important role in the tissue damage during the inflammation action. This analysis is done by triplicate, and the results were calculated through the mean and standard deviation to fine the variables between the samples as shown in Table 8 and Figure 15.

4. Conclusion

The nanoparticle screening confirms the presence of phytochemicals such as carbohydrates, saponins, tannins, flavonoid, alkaloids, and steroids. Characterization of NPs gave the structures and size for XRD evaluated by the Debye-Scherrer formula, and for FTIR, the presence of functional group distinguishes by value of peaks. For microbial studies, the result of antibacterial and antifungal activities was observed by the zone of inhibition. Other potentials of copper oxide nanoparticles were also evaluated like antioxidant, antidiabetic, and anti-inflammatory activity and antiproteinase action. Therefore, the mechanism behind its action is yet to be researched, but these nanoparticles can also be an agent which fills the criteria of eco-friendly, nontoxic, and easy production in larger scale.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Authors’ Contributions

SR designed the research; and SR and SM carried out research; and SM, SV, MP, DA, and KA wrote and corrected the manuscript.

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