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X ray diffraction (XRD) analysis and evaluation of antioxidant activity of copper oxide nanoparticles synthesized from leaf extract of *Cissus vitiginea*

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Abstract

Nanoparticles have a diameter of up to 100 nm and a higher surface-to-volume ratio, enabling more active surface atoms to contribute to implementations and improve material properties. In nanoparticle preparation, the ability to control particle size, shape, and morphology is important. The most important tool for studying nanomaterials is XRD, it is a vital characterization tool in solid-state chemistry and materials science. For any compound, XRD is a simple method for determining the unit cell's size and shape. This study explains how copper oxide nanoparticles are formed in *Cissus vitiginea* leaves. The antioxidant function and XRD study of the synthesized CuONPs were also investigated. According to the XRD results, the copper oxide nanoparticles formed by reducing

Cu^{2+} ions by *Cissus vitiginea* leaf extract are crystalline in nature. CuONPs have an average crystalline size of ~32.32 nm, according to the Debye-Scherrer formula. CuONPs have higher antioxidant activity than plant extract and are closest to ascorbic acid in terms of standard.

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Keywords

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ABSTRACT

Nanoparticles have a diameter of up to 100 nm and a higher surface-to-volume ratio, enabling more active surface atoms to contribute to implementations and improve material properties. In nanoparticle preparation, the ability to control particle size, shape, and morphology is important. The most important tool for studying nanomaterials is XRD, it is a vital characterization tool in solid-state chemistry and materials science. For any compound, XRD is a simple method for determining the unit cell's size and shape. This study explains how copper oxide nanoparticles are formed in *Cissus vitiginea* leaves. The antioxidant function and XRD study of the synthesized CuONPs were also investigated. According to the XRD results, the copper oxide nanoparticles formed by reducing Cu2 + ions by *Cissus vitiginea* leaf extract are crystalline in nature. CuONPs have an average crystalline size of ~32.32 nm, according to the Debye-Scherrer formula. CuONPs have higher antioxidant activity than plant extract and are closest to ascorbic acid in terms of standard.

Selection and peer-review under responsibility of the scientific committee of the 1st International Conference on Computations in Materials and Applied Engineering – 2021.

1. Introduction

Nanoparticle biosynthesis offered an appealing alternative to chemical synthesis methods. They predict that dyes will be used to treat contaminated water sources in the future, and they are also a promising candidate for a variety of medical applications Fig. 1.

A bottom-up 'green' route can be used to make silver nanoparticles (AgNPs). They are spherical in shape and range in size from 20 to 30 nm. Several pathogenic bacteria were demonstrated by antibacterial and synergistic activity with conventional antibiotics. Nanoparticles can enhance the antibiotic potential and also treat bacterial infections. The photosynthesis of U. dioica extract AgNPs is found to be cost-effective, straightforward, and environmentally friendly [1].

Green synthesized AgNPs were tested for antimicrobial activity against a variety of microorganisms. This research demonstrated that biomaterials could be used to synthesise silver nanoparticles using green chemistry principles.

A new electrolysis of silver nanoparticles using AgNO3 for metal precursors, which is economical and environmentally friendly, is mentioned in this review. Ag nanoparticles were detected in microbiology experiments to be effective against E.coli and B. megaterium bacteria. The actual surface area (SSA) is 24 m2 per gramme. The particles measure 24 nm. Bacterial SSA studies show that antimicrobial agent reactions have a major role to play. This method provides for the synthesis without the need of additional agents of nanopowder tunable particle size at room temperature to be safe, non-toxic, environmentally friendly and effective. Two

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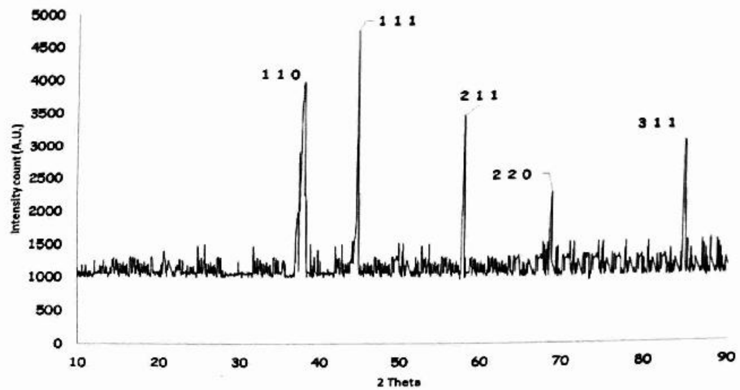

Fig. 1. XRD pattern of copper oxide nanoparticles synthesized by *Cissus vitiginea* leaf.

different methods have been used to investigate SSA of Silver Nanoparticles and the higher SSA has been found to lead to a rise in antibacterial activity of silver nanoparticles. The synthesised silver nanopowder can be used in a number of applications [2].

One of the most common and interesting nanomaterials is silver nanoparticles (AgNPs). More research is required to fully understand the synergistic impact of the two cytotoxic agents simultaneously. It will aid in developing a novel device with multiple components that work together to treat different forms of cancer. Studies are unavoidable to ensure the biosafety of AgNP usage in humans. Nanotechnology-based therapy could outperform current treatments. It should be able to overcome the limitations of the current system [3].

Efforts were made to conduct pharmacognosy studies on Cissus. Tannins, protein, and steroid were found in most of the extracts in the three primary forms of plant matter: leaf, root, and stem. Fruits are edible, as well as claimed to be useful as medicinal. In various developed and developing countries, primary healthcare is in great demand due to its numerous medical and biological actions and lower costs [4].

Copper ions (Cu + ions) can be valuable in several therapeutic applications. Biosynthesize copper-plate can be done successfully using plant extract from C. arnotiana. Gram-negative bacteria exhibited the most antibacterial properties because of their very thin peptidoglycan layer and CuN Ps. We are excited about the results because they lay the groundwork for an entirely new field of study in bacterial and antioxidant activity nanotechnology. In this manner, CUPS is a treatment for ulcers.

Silver nanoparticles were created by chemically reducing silver nitrate solution with ethanol. Nanoparticle synthesis is critical to the advancement of contemporary science. According to this research, the particles are mainly spherical in shape and have sizes of less than 18 nm. In the UV–Vis absorption spectra, the surface plasmon resonance peak exhibits maximal absorption at 422 nm. The presence of the SPR peak is the primary indicator of the formation of metal nanoparticles.

Conventional methods of decontamination are not appropriate for treating spices. The thermolability of many essential oil components means that heat cannot be used. The decontamination of high volumes due to its short penetration is not successful by UV radiation. The best method for microbial decontamination of the spices and plants without altering the condiment consistency seemed to be β-irradiation. Commercial irradiation in over 40 countries has already been used in the US, France, Holland, Belgium and China with prescribed doses [5].

Nanoparticles of copper (II) oxide (or copper oxide nanoparticles) have gained notoriety for their chemical and physical properties. Useful for gene therapy are the relatively rapid growth, transparency, and small zebrafish embryos' small size. It's been suggested that the zebra fish is an in-vitro experimental model for new drug discoveries. The speed of their proliferating tissues allows for the assessment of new medications. The resulting information depends on the size and number of NP. Antioxidants, anticancer, anti-inflammatory, and antimicrobial activities, as well as antiviral activity Silver, was found to be an antimicrobial against several pathogenic microbes in combination with other ingredients in it. Featured in nanotubes can enhance the active surface and initial optical, chemical, and electrochemical properties. A new composite of copper and bismuthide is reported to have higher catalytic activity than monometallic copper for copper bismuthide applications [6].

The Ti plant is found in many agricultural and ornamental farms. Anticancerous claims are made about this plant. Green syntheses of nanoparticles have gained attention in the last decade as a way to create nontoxic compounds over the last decade. In the present investigation, researchers synthesized copper nanoparticles using water extracts of one plant, Cucumaria frondosa, which is also known as sea cucumber. It was determined by the Free Radical Catalyzing Determination Test that the antioxidant properties of the nanoparticles [7].

Survey of the *Cissus vitiginea* plant leaves focussing on the phytochemical components, antimicrobial activity and trace metal levels. Leaves from the district of Tiruchirappalli, Southern India, have been collected. A new pharmaceutical drug could be isolated and identified as a bioactive compound accountable for these antimicrobial activities. Antimicrobial sensitivity to C. Vitiginea has been tested for bacterial and fungal strains. The methanol extracts from the leaves of the plants had a higher inhibition zone than the fungal strain. This study showed that the leaves contain all the phyllosmetics components. In the coming years, the plant could be a fungal antibiotic alternative [8].

Tissue extracts of Cissus affinis, flavonoids, polyphenols, steroids, saponins, glycosides, terpenoids, and triterpenes were found in leaves of the Cissus plant. Inorganic elements found in Cissic found the presence of calcium, sodium, potassium, sulphate, bicarbonate, boronate, and phosphates, but did not mention any of Chloride or This type of treatment can be applied to diseases that have not been linked to vitamin deficiency, such as hypertension, diabetes, and cancer for human health. Phytochemicals are biologically active chemicals, present in all plant matter, which assist the human body in healing and general well-being. They keep plant life going and protect their colour, scent, and taste. The conclusion of the study stated that Cissus viticella had positive phytochemical and mineral content. Useful human disease-related diseases such as cardiovascular disease, diabetes, hypertension, and cancer can be supplemented with this. Our investigation has determined that CISSus Vitis extract can be a rich source of phytochemicals [9].

Nano-copper was discovered commercially to be produced in the fruit fungus Cephalotyceria. Synthesised nano-copper was examined for urinary tract infections. We observed that the particles effectively killed pathogenic bacteria but didn't significantly hamper the urinary tract activity. They had a significant antimicrobial and antioxidative effect against pathogenic strains of urinary tract infection (UTI). As shown by these experiments, green-related copper may well be a promising option for treating infected urinary tract bacteria [10].

Cissus is a genus of a couple of dozen different plant species, widely distributed across the world, that have been used in traditional medicine in various parts of the world for a variety of ailments. Further investigation is required to assess the concentration and determine the precise mechanisms involved. These plants could be useful in treating diabetes and osteoporosis and a source for drug discovery and research [11].

Four-fifty percent of allopathic medicines are derived from chemical ingredients. Many species of the genus Cissus have been used traditionally to treat a variety of ailments in various regions throughout the world. Additional studies are needed to examine and quantify their role. The lack of knowledge of the long-term impacts of this study is going to have on the ecosystem has brought my work to a screeching halt [12].

The operational parameters that are critical to the synthesis of silver nanoparticles were discussed in this chapter. The synthesis is influenced by the concentration, volume ratio, contact time, temperature, and pH. It can be concluded that relevant nanoparticle research is based on both operating conditions and excellent characterization. The surface plasmon resonance absorption band can be determined with UV–Vis spectroscopy. The functional types are determined by FTIR and the morphology is determined by the SEM and TEM.

Traditionally, wet chemicals were used to make silver nanoparticles and often dangerous and inflammable chemicals are in use [13].

2. Materials and methods

2.1. Synthesis of copper oxide nanoparticle (CuONPs)

"2.8 g copper acetate monohydrate was dissolved in 500 ml deionized water and magnetically stirred at room temperature for 5 min in a standard reaction mixture. Following that, an aqueous extract of *Cissus vitiginea* leaves was added dropwise when stirring; as soon as the leaves extract comes into contact with copper ions, the blue colour of copper ions changes to green. The resulting green mixture was held at room temperature while being stirred. Ghidan et al., (2016) [6] observed that after 10 min, the blue mixture began to transition to a green suspended mixture, suggesting the development of water-soluble monodispersed copper oxide nanoparticles."

2.2. Leaves extract preparation

"Thanjavur has been used to collect good *Cissus vitiginea*'s fresh leaves. The leaves were washed several times with water to remove dust particles before being dried in the shade for two weeks to remove any residual moisture. The leaves of Cissus vitilininea were collected aqueously in a 500 ml glass beaker with 10 g of dried fine powder in 400 ml of sterile distilled water. The solution was boiled for 10 min or until the colour of the watery, brown-yellow solution changed. The blend was then allowed to cool down to room temperature before filtering it with Whman No. 1 filter paper and centrifuged to extract biomaterials for about 5 min at 1200 rpm. The extract was kept at room temperature to be used in future research."

2.3. Characterization of nanoparticles

2.3.1. X-ray diffraction method and electron microscopy

Using Cu Ká radiation, the phase evolution of calcined powder and sintered samples was analyzed using an XRD technique. The voltage and current of the generator were set to 40KV and 30 mA, respectively. In continuous scan mode, the Cu sample was scanned in the 2è ranges 15 to 700 °C. The scan speed was 0.03 s per second. The diffracted intensities were noted from 35 to 90° 2θ angles The TEM study was done by CM30–Philips at functioning voltage of 80 kV. The energy dispersive x-ray spectroscopy (EDS), attached to the SEM, conducted a compositional analysis of the sample.

2.4. DPPH radical-scavenging activity

Shimada approach is used to assess DPPH radical-scavenging operation. At various concentrations (20, 40, 60, and 80 g/ml), a two ml aliquot of DPPH methanol solution (25 g/ml) was applied to a five ml sample solution. The mixture was vigorously shaken before being left at room temperature for 30 min in the dark. The absorbance was then measured in a spectrophotometer at 517 nm. The reaction mixture's lower absorbance demonstrated higher free-radical scavenging activity.

3. Results and discussion

3.1. Synthesis of copper oxide nanoparticles

The photosynthesis of CuO nanoparticles was investigated using an aqueous leaf extract of *Cissus vitiginea*. During the visual test, copper acetate that had been incubated with leaf extract changed colour from blue to green, while copper acetate that had not been incubated with leaf extract did not change colour. The presence of green colour in leaf extract treated flasks is a clear indication of CuO nanoparticles' formation, as stated by Gnanasundaram and Velavan's previous research (2020). According to Gnanasundaram and Balakrishna (2018), the efficiency of *Cissus vitiginea* leaf extract in the faster synthesis of CuO nanoparticles with a variety of fascinating morphologies is due to the presence of various classes of phytochemicals such as polyphenols, anthraquinones, terpenoids, phenolics, reducing sugars, anthrones in the leaf extract and, favonoids.

3.2. XRD pattern of CuONPs synthesized from Cissus vitiginea leaf

Powder XRD is one of the most popular methods used by mineralogists and solid-state chemists to investigate unknown materials' physicochemical composition. For any compound, XRD is a simple method for determining the unit cell's size and shape.

Powder diffraction methods help qualitative, quantitative, and other types of analysis. Peak Locations reveal translational symmetry, specifically the size and shape of the unit cell. On the other hand, Peak Intensities provide details on electron density within the unit cell, i.e. where the atoms are located [10]. Theivasanthi and Alagar, Theivasanthi and Alagar, Theivasanthi and Alagar,

Several Bragg reflections with 2ø values of 38.02, 44.56, 57.68, 68.51 and 84.540 indicate the (110), (111), (211), (220), and (311) reflections of metallic copper specify the cubic crystalline face-centred cubic structure of copper. Here, the constant is 40.8 (136.9–96.1 = 40.8). Biosphere crystallisation on the nanoparticle surface could activate the unassigned peaks. Macromolecules were most likely responsible for copper ion lowering and exacerbated the plant extract's peak enlargement and noise. The XRD pattern has therefore shown that the nanoparticle produced in this synthesis is crystalline in copper oxide. There are other unallocated peaks and Bragg peaks that show copper nanocrystals, which indicate that the bio-organic process crystallises on copper nanoparticles' surfaces. The expansion of the pits is primarily due to the small size of the particle. Table 1 and Table 2 contain indexed data.

3.3. Particle size calculation

In this research, the average particle size was determined in light of the peak at degrees by using Debye-Scherrer formula Sun *et al.*, (2002) [11], Nath *et al.*, (2007) [12], Nath *et al.*, (2008) [13], Branauer *et al.*, (1938) [14].

$D = 0.9\,\lambda / \beta \cos\theta$

Where,

D = particle diameter size.

θ = diffraction angle

β = Full width at half maximum,

λ = wave length of X-Ray (0.1541 nm),

The average calculated crystalline size by Debye-Scherrer eq has been 32.32 nm.

The biosynthesized CuONPs of the leaf extract was tested on an X-ray diffraction pattern. Indexing is the process by which cell dimensions from top positions are determined. This is the first step in the analysis of diffraction patterns. The Miller Indices (hkl) should be assigned to each Cullity peak (1978) to index a pattern of polvo diffraction [15]. A Copper Nanopowder Sample XRD analysis of the Goniometer was performed. For the 2-fold range, data were collected from 10 to 90° with the step of 0.03°. The powder diffraction pattern indexing was performed, with the first phase assigned to Miller indices (*hkl*) for each top. Two different methods have been used to index, and Table 1 and Table 2 are used for the data. The same result is achieved with these two methods. A dividing constant must be found in Table 1. The 3rd column values become whole (approximately). Here, the constant is 40.8 (136.9–96.1 = 40.8). Moreover, the high intense peak for FCC materials is generally (111) reflection, which is observed in the sample.

Table 1
Copper oxide nanoparticles synthesised by *Cissus vitiginea* leaf XRD pattern simple Peak indexing process.

1000 × Sin 2θ	Peak Position 2θ	1000 × Sin 2θ/ 40.8	Reflection	Remarks
96.1	38.02	2.3553	2	110
136.9	44.56	3.3553	3	111
230.4	57.68	5.647	6	211
313.6	68.51	7.6862	8	220
448.9	84.54	11.0024	11	311

Table 2
The grain size of copper oxide nanoparticle.

2 θ of intense peak (deg)	Miller indices (*hkl*)	θ of the intense peak (deg)	FWHM of intense peak (β)	Size of the particle (D) nm
38.02	110	19.01	0.3314	24.5773
44.56	111	22.28	0.3888	21.9349
57.68	211	28.84	0.5033	18.8878
68.51	220	34.255	0.5978	14.2526
84.51	311	42.27	0.7377	81.968
Average size of particles				32.3241

X-Ray Diffraction pattern confirmed the successful synthesis of copper nanoparticles with a shell of copper oxide; wherein the main diffraction peaks characterize the elemental copper were detected at 2θ = 38.08, 44.56, 57.68 and 68.51 which correspond to the (110), (111), (211) and (220) crystal faces of copper and compared with the standard powder diffraction card of JCPDS, copper file No. 01–078-2076 Berra *et al.*, (2018) [16]. It is also noteworthy that there are other peaks at 2θ = 38.02 and 68.51that are characteristic for Cu_2O and both peaks were attributed to the presence of a Cu_2O shell covering the copper core Johan *et al.*, (2011) [17], Zhu *et al.*, (2012) [18]. Whereas the peaks at 2θ value of 29.85°, 36.44°, 42.29°, and 61.41° correspond to the planes (110), (111), (200), and (220) of cubic crystal structures of Cu_2O much well with the standard as JCPDS Card No. 01–078-2076.

Fig. 2 exhibits TEM images of the synthesized copper oxides nanoparticles. It is clearly shown that in general the particles are roughly spherical and irregular shaped, which are free from agglomeration. The majority of the CuO and Cu2O nanoparticles in the mixture are spherical in nature; additionally, the particles are agglomerated to form foam like many particles. For Cu_2O nanoparticles is observed that there is more than one shape (spherical nanoparticles) as depicted in TEM image. The nanoparticles become greater in dimension having the form of foam like bunch.

3.4. *In vitro* antioxidant activity of *Cissus vitiginea* leaves and copper oxide nanoparticles

The DPPH radical is stable since a spare electron is delocalized over the molecule, preventing dimer formation. The DPPH radical scavenging power assay is used to assess antioxidants' capacity to quench the DPPH radical. When DPPH is decreased to its non-radical form by antioxidants, the dark purple colour is lost. DPPH is stable organic nitrogen centred free radical with a dark purple colour that becomes colourless when reduced to its nonradical form. The model system of DPPH radicals is commonly used to investigate the scavenging behaviours of various natural compounds. The colour of the reaction mixture changes from purple to yellow as the DPPH radical is scavenged, with decreasing absorbance at 517 nm. (2009) [19].

DPPH radical scavenging activity of *Cissus vitiginea* leaves extract, CuONPs and standard as ascorbic acid are presented in Table 3 and Fig. 3. The half inhibition concentration (IC_{50}) of *Cissus vitiginea* leaves extract, CuONPs and ascorbic acid were 50.51, 45.29 and 41.33 μg/ml respectively. The CuONPs exhibited a significant dose dependent inhibition of DPPH activity (Table 3) as compared to *Cissus vitiginea* leaves extract. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentrations. CuONPs has potential antioxidant activity than *Cissus vitiginea* extract and near to standard. Antioxidant activity CuONPs

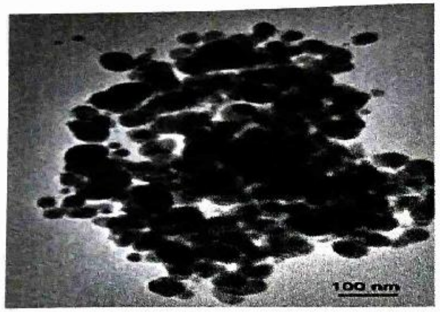

Fig. 2. TEM analysis of copper oxide nanoparticles synthesized by *Cissus vitiginea* leaf.

Table 3
DPPH radical scavenging activity of *Cissus vitiginea* leaves extract, CuONPs and Ascorbic acid at different concentrations.

Concentrations (μg/ml)	% of inhibitions		
	Cissus vitiginea	CuONPs	Std. (Ascorbic acid)
20	20.26 ± 1.41	24.22 ± 1.69	25.99 ± 1.81
40	31.71 ± 2.21	40.96 ± 2.86	48.01 ± 3.36
60	63.43 ± 4.44	68.28 ± 4.77	73.56 ± 5.14
80	82.37 ± 5.76	86.78 ± 6.07	90.31 ± 6.32
IC_{50} (μg/ml)	50.51	45.29	41.33

Values are expressed as Mean ± SD for triplicates.

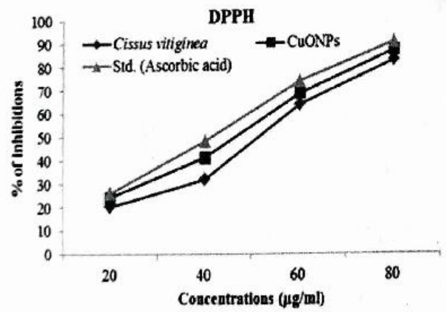

Fig. 3. DPPH scavenging activity of *Cissus vitiginea* leaves extract, CuONPs and Ascorbic acid at different concentrations.

is better than plant extract and nearest to standard as ascorbic acid.

4. Conclusion

The green synthesis is eco-friendly, thus cheap and CuONPs can be produced. The leaf extract from *Cissus vitiginea* is used as a reduction and stabilising agent. CuONPs confirmed green-synthesized Copper oxide nanoparticles' surface plasm resonance. The results of X-ray diffraction show that Cissus-vitiginea leaf extract copper oxide nanoparticles formed by reducing Cu^{2+} are crystalline in nature. According to the Debye-Scherrer formula, CuONPs average crystalline sizes are ~32.32 nm. In this analysis, X-ray diffraction confirmed the crystalline nature of CuONPs. Cu2O nanoparticles are shown to have more than one form, as shown in TEM picture (spherical nanoparticles). CuONps antioxidant activity confirmed in the present study.

CRediT authorship contribution statement

Minakshi A. Thakar: Investigation, Writing - original draft. **Subhesh Saurabh Jha:** Conceptualization, Writing - review & editing, Supervision. **Khongdet Phasinam:** Formal analysis, Data curation. **Ravi Manne:** Conceptualization. **Yaser Qureshi:** Writing - review & editing. **V.V. Hari Babu:** Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Further Reading

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