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Green Synthesis, Characterization And Antioxidant Activity Of Copper Oxide Nanoparticles Using *Annona Squamosa* Leaf Extract

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Abstract
The present study focuses on the green synthesis of Copper Oxide (CuO) nanoparticles using the ethanol leaf extract of *Annona squamosa* as a bioreducing and stabilizing agent. This eco-friendly approach avoids the use of hazardous chemicals commonly employed in conventional synthesis methods. The formation of CuO nanoparticles was initially confirmed by the appearance of a characteristic surface plasmon resonance peak in the UV–Visible spectrum around 270–280 nm. Further structural and morphological characterization using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) revealed the formation of monoclinic CuO nanoparticles with crystalline nature and nanoscale dimensions. The green synthesis method demonstrates a simple, cost-effective, and sustainable route for producing CuO nanoparticles with potential applications. X-ray diffraction analysis confirmed the crystalline nature of the synthesized nanoparticles with an average crystallite size of 25.81 nm, while scanning electron microscopy revealed particle sizes ranging from 20 to 80 nm. The nanoparticles exhibited notable antioxidant activity, with an IC50 value of 21.117 µg/mL, indicating strong free radical scavenging potential. It was found that *Annona squamosa* plant leaves are a suitable alternative for the easy and green synthesis of CuO nanoparticles. According to the results of this investigation, green synthesized CuO NPs with *Annona squamosa* leaf extract may be used in biomedicine as a replacement agent for biological applications.

Keywords: *Annona squamosa*, Copper oxide nanoparticles, green synthesis, characterization

Introduction

Nanotechnology is one of the most active fields of research in materials science [1], and may be defined as an intersection of technologies including different domains [2]. It is a great favour for humanity because its importance has covered the way for numerous applications in therapeutics [3], catalysis [4], microelectronics and biological sensors [5].
Nanotechnology is one of the essential areas of research in modern sciences. The field of nanotechnology is expanding very rapidly, creating an incredible impact on human life including pharmaceutical, food, health, chemical industry, electronics, energy science, cosmetics, space industries, and environmental sciences [6]. The interest in nanotechnology derived products is rapidly increasing. Nanotechnology that is the inventive innovation in the present situation can enhance human well-being and also create a great effect on the improvement of human health [7].
Nanotechnology is an important branch in the major field of science. It concerned with the synthesis and development of various types of nanoparticles with size range from 1 to 100nm [8].
Biological synthesis of nanoparticles utilizes a bottomup approach in which synthesis occurs with the help of reducing and stabilizing agents. Phytochemicals present in the plant extracts reduce metal ions to nanoparticles readily at room temperature and pressure [9]. The green synthesis method of CuO NPs with medicinal plants is eco-friendly, cost effective and stable.
Copper oxide nanoparticles (CuO NPs) are p-type semiconductor nanomaterials with a narrow band gap (~1.2–1.9 eV), making them attractive for various technological and biomedical applications. Due to their high surface area, small particle size, and unique electronic properties, CuO NPs exhibit enhanced catalytic, antimicrobial, antioxidant, and photocatalytic activities compared to bulk copper oxide [10]. In recent years, green synthesis approaches using plant extracts have gained attention as eco-friendly alternatives to conventional chemical methods, where phytochemicals act as reducing and stabilizing agents [11]. These nanoparticles have found applications in sensors, energy storage devices, environmental remediation, and biomedical fields.
In this study, green synthesized copper oxide nanoparticles using *Annona squamosa* leaf extract was investigated. Characterizations of the obtained nanoparticles were analyzed by standard techniques such as UV-Vis, FT-IR, XRD, and SEM.

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Materials and Methods

Plant material and Extraction

Leaves of *Annona squamosa* were collected from local fields in the region of Thovalai, Kanyakumari. Fresh leaves were washed and dried in a shade under room temperature for 15 days. The extraction of plant materials was carried out following the maceration method. 25 grams of the powdered material was then extracted using ethanol in Erlenmeyer flasks (250 ml). Before its use, the mixtures were kept for 72 hours in tightly sealed vessels at room temperature.

Synthesis of copper oxide nanoparticles

Copper oxide nanoparticles were synthesized through a reduction of copper sulphate by the phenolic compounds of leaf extract. The reaction mixture was prepared by adding 50mL of the plant extract to 100mL of 0.1M $CuSO4.5H2O$ solution in a 250 mL beaker. The reaction was carried out using magnetic stirrer for 2 hours. The formation of copper oxide was confirmed by a colour change from green to dark brown [12]. The brown solid product was collected by centrifugation at room temperature and washed several times with absolute ethanol and distilled water. The product was dried over night at 80 °C then is heated in a furnace at 400°C for 2 hours. The annealed powder considered the samples of our study.

Characterization of copper oxide nanoparticles

The synthesized CuO-NPs were characterized using various analytical techniques to confirm their formation, structural properties, functional groups, crystalline nature, and surface morphology. UV–Visible spectroscopy was employed to verify nanoparticle formation, Fourier Transform Infrared (FTIR) spectroscopy was used to identify the biomolecules involved in synthesis and stabilization, X-ray Diffraction (XRD) analysis was carried out to determine the crystalline structure, and Scanning Electron Microscopy (SEM) analysis was performed to examine the morphology and particle size of the synthesized nanoparticles.

Results and Discussion

UV-Vis Spectroscopy

The UV-Vis spectra of copper oxide nanoparticles synthesized using *Annona squamosa* leaf extract are shown in Fig. 1. The spectrum shows that there is a strong absorbance of the UV rays between the wavelength 200-300nm. This reveals the presence of CuO nanoparticle in the sample [13]. The peak formed at 271 nm is due to surface plasmon absorption of CuO nanoparticles. The combined oscillation of the free conduction band electrons present in the metal oxides which is excited by the incident UV radiation is the reason for surface plasmon absorption. This type of resonance is seen when the wavelength of the incident light far exceeds the particle diameter [14].

Similar observations of absorption peaks around 270–280 nm for CuO nanoparticles synthesized via green routes have been reported in previous studies, confirming the successful formation of CuO nanoparticles [15,16].

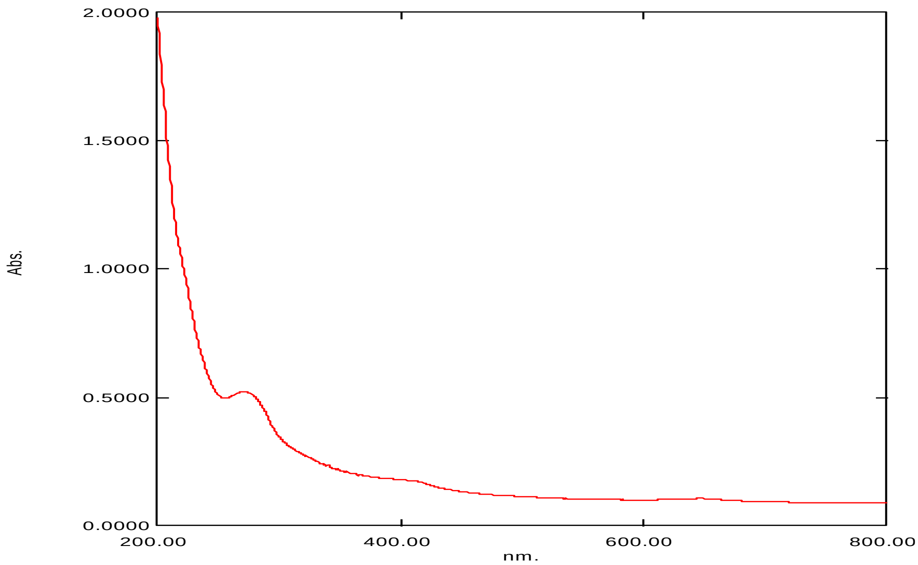

Figure 1: UV-Vis absorption spectrum of Copper oxide nanoparticles

Fourier-transform infrared spectroscopy

FTIR analysis of the green synthesized CuO nanoparticle is shown in Fig. 2. Different vibrational bands between 500 and 4000 cm^{-1}show functional groups accountable for the reduction of copper. The distinct band observed at 615 cm^{-1} corresponds to Cu–O stretching vibrations, confirming the formation of copper oxide nanoparticles [17,18]. The broad peaks at 3452–3471 cm^{-1} are attributed to O–H stretching of hydroxyl groups from alcohols and phenolic compounds,

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while the absorption at 2945 cm⁻¹ represents C–H stretching of aliphatic groups [19]. The band at 1635 cm^{-1} corresponds to C=O stretching or amide I vibrations of proteins, suggesting their role in the capping and stabilization of nanoparticles. Peaks around 1107–1190 cm^{-1} indicate C–O stretching of alcohol or ether groups, confirming the presence of bioactive compounds on the nanoparticle surface [20]. The weak absorptions near 2100–2375 cm^{-1} are due to atmospheric CO_2, while the higher-wavenumber peaks (3776–3973 cm^{-1}) may arise from overtone or free O–H vibrations [21]. Overall, these results confirm the successful biosynthesis of CuO nanoparticles stabilized by phytochemicals from plant extract.

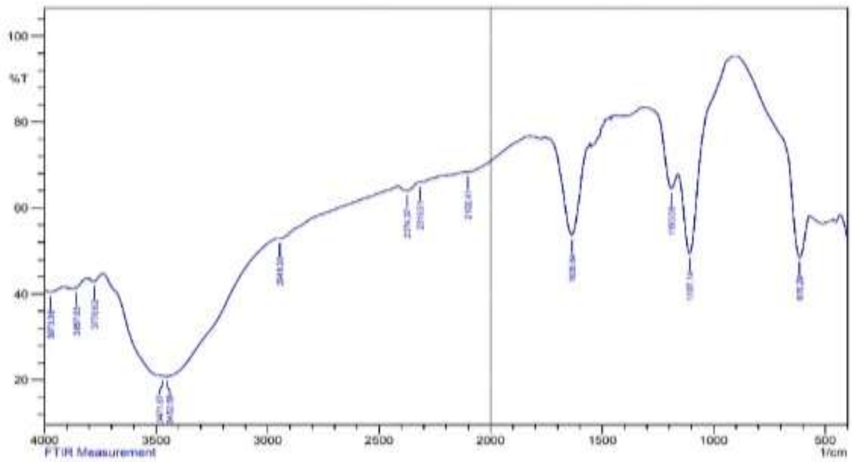

Figure :2 FTIR spectrum of copper oxide nanoparticles

X-ray diffraction (XRD) analysis

The XRD technique was used to determine and confirm the crystal structure of the nanoparticles. XRD analysis showed a series of diffraction peaks at 2θ of 32.32, 35.68, 38.80, 48.96, 53.67, 58.46, 61.46, 66.30, 68.12, 72.63 and 75.21 which were assigned to (110), (-111), (111), (−202), (020), (202), (-113), (022), (220), (311) and (004) planes respectively. Those planes accord well with the (JCPDS Card No. 48-1548 [22]. The XRD spectrum clearly suggested the crystalline nature of the CuO NPs synthesized from leaf extract of *Annona squamosa*.

The average crystallite size of CuO nanoparticles was calculated using Scherrer formula, $D=0.9\ \lambda/\beta \cos\theta$, where λ is the wavelength of X-ray radiation, β is the full width at half maximum (FWHM) of the peaks at the diffracting angle θ [23]. It was found to be about 25.81 nm indicating its nanocrystalline nature.

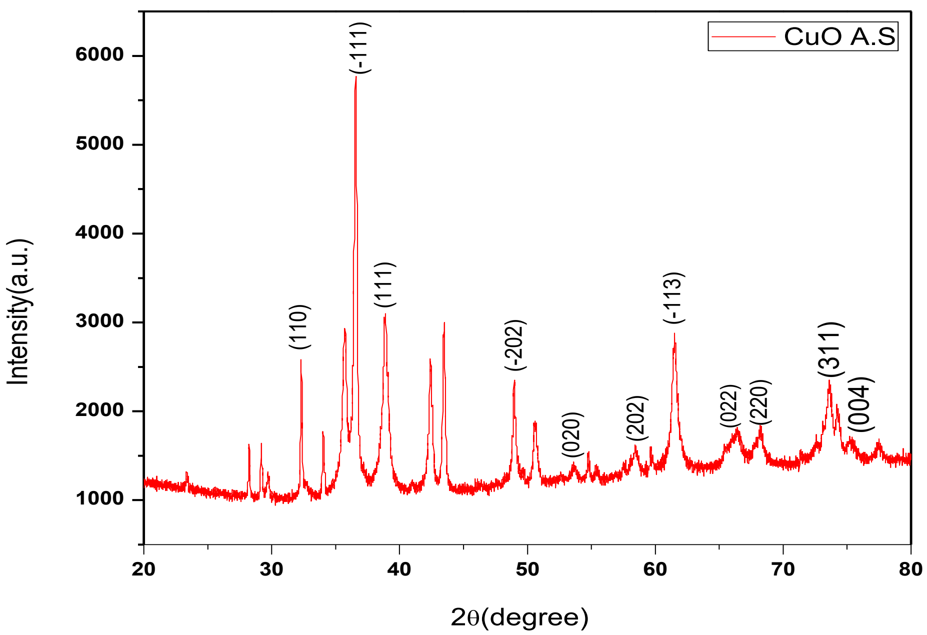

Figure 3: XRD pattern of copper oxide nanoparticles

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Scanning Electron Microscopy (SEM) analysis

SEM confirmed the morphology of the synthesized CuO nanoparticles. Figure 4 shows the morphological form of CuONPs. From the SEM image, it is observed that the CuO nanoparticles are in highly collected form and have almost spherical morphology. The particle size of synthesized NPs was ranging from 20 to 80 nm, measured by using ImageJ software. The results are consistent with XRD results.

The SEM image of copper oxide (CuO) nanoparticles shows nearly spherical particles with slight aggregation, confirming the formation of nanosized CuO. The particles appear to be in the nanometer range, showing some degree of agglomeration which is common due to the high surface energy and strong interparticle attraction of nanosized materials. The surface texture indicates a rough and porous nature, suggesting the successful formation of CuO nanoparticles. Such morphology enhances the surface area, which is beneficial for various catalytic and biological applications. Similar surface features of CuO nanoparticles have been synthesized using plant extracts exhibited spherical morphology with slight agglomeration due to particle interaction [24].

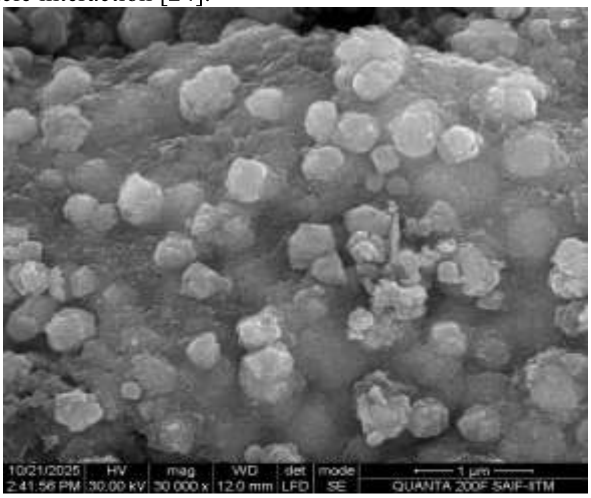

Fig 4: SEM image of Copper oxide nanoparticles

Antioxidant activity

DPPH radical scavenging activity

Oxidative stress caused by excessive free radicals is associated with the development of various chronic diseases, including cancer, diabetes, and cardiovascular disorders. Therefore, the evaluation of antioxidant activity is essential to determine the free radical scavenging potential of bioactive compounds and nanomaterials. In the present study, the antioxidant activity of plant-mediated CuO nanoparticles synthesized using *Annona squamosa* leaf extract was evaluated using the DPPH radical scavenging assay and compared with ascorbic acid as the standard antioxidant. The results are presented in Table 1.

Table 1: Antioxidant activity of copper oxide nanoparticles

Concentration	**% of inhibition**	
	Ascorbic acid (Standard)	**Annona squamosa**
20	89.42	46.44
40	91.64	57.80
60	92.52	61.84
80	93.43	65.33
100	94.81	68.18
IC50 (µg/mL)	**11.18**	**21.117**

The DPPH radical scavenging activity of *Annona squamosa* extract was evaluated and compared with ascorbic acid as a standard antioxidant. Ascorbic acid exhibited strong antioxidant activity, showing % inhibition values ranging from 89.42% to 94.81% at concentrations between 20 to 100 µg/mL.

The *Annona squamosa* extract also demonstrated a concentration-dependent increase in DPPH radical scavenging activity, with % inhibition increasing from 46.44% at 20 µg/mL to 68.18% at 100 µg/mL. This gradual increase indicates effective hydrogen-donating and free-radical scavenging capacity of the extract [25].

The *Annona squamosa* extract exhibited an IC_{50} value of 21.117 µg/mL, indicating strong free radical scavenging activity, which may be attributed to the presence of bioactive phytochemicals such as phenolic and flavonoid compounds [26].

The results confirm that *Annona squamosa* possesses significant antioxidant potential and can act as a natural source of free-radical scavengers, supporting its possible application in pharmaceutical and nutraceutical formulations.

Conclusion

The present study reports the successful synthesis of copper oxide nanoparticles (CuO NPs) mediated by *Annona squamosa* leaf extract. The study successfully demonstrated the biosynthesis and characterization of *Annona squamosa* extract-based nanoparticles using various analytical techniques. UV–Vis spectroscopy confirmed the formation of by exhibiting characteristic absorption peaks. FTIR analysis revealed the functional groups, indicating the presence of phenolics, flavonoids, and other bioactive compounds responsible for stabilization and antioxidant activity. XRD patterns confirmed the crystalline nature of the synthesized material, while SEM images provided detailed insights into the surface morphology and particle size. Furthermore, the extract exhibited significant antioxidant activity with an IC_{50} value of 21.117 µg/mL, reflecting its strong free radical scavenging potential.

Overall, the findings highlight the phytochemical richness and biological potential of *Annona squamosa*, suggesting its application in pharmaceutical, nutraceutical, and biomedical fields.

Author contributions

N. Megilda carried out the experiments, collected and analyzed the data, and wrote the original draft of the manuscript. Dr. S.P.R. Kalaikathir supervised the research work and provided overall guidance.

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