Research Article

Green Synthesis of CuO Nanoparticles by Using *Cynodon dactylon* Characterization and its Antioxidant Activity Evaluation

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ABSTRACT

The study aims to synthesise copper oxide nanoparticles (CuO NPs) using *Cynodon dactylon* plant extract at room temperature. This method is completely green and free from toxic and harmful solvents. A simple method for green synthesis using an aqueous extract of *Cynodon dactylon* leaf as a reducing and stabilising agent resulted in CuO NPs being rapidly synthesised with copper sulphate dehydrate ($CuSO_4.5H_2O$) within 4 hrs. The green synthesised CuO NPs were characterised using physicochemical techniques, viz., X-ray diffraction (XRD) and scanning electron microscope (SEM), coupled with X-ray energy dispersive spectroscopy (EDX), UV-vis spectroscopy, and Fourier-transform infrared spectroscopy (FT-IR). UV-vis spectroscopy demonstrated the existence of CuO nanoparticles. The FTIR spectra of the control (leaf extract) and synthesized CuO NPs identified the functional groups of the active components. SEM images brought out that the particles were spherical in shape, and the size was found to range under 70 nm. Further, the synthesized CuO NPs were tested for antioxidant activity by the in vitro method. The nanoparticles were found to have antioxidant activity against free radicals by using DPPH and H_2O_2 in an in vitro method, and the absorbance value of different concentrations of prepared CuO NPs was determined by using a UV-vis spectrophotometer. The IC50 value of biologically synthesised CuO NPs is found to be significantly effective against oxidative stress and less toxic than the precursor material.

Keywords: *Cynodon dactylon*, green method, CuO nanoparticle, antioxidant activity.

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INTRODUCTION

Nanotechnology is one of the fastest-developing technologies, and its products are very useful in all fields because of their small size (9–10 nm) and large surface area. In comparison to analogous bulk materials, nanoparticles have a higher surface-to-volume ratio and a higher concentration of partly coordinated surface sites. Elastic, geometrical, and electrical characteristics interact strongly, giving nanoparticles their distinctive properties. The result of these features is often improved physical and chemical properties compared to bulk materials. According to the estimations of the National Science Foundation (NSF), Alexandria, USA, the global market for nanotechnology-based products would reach three trillion USD by the year 2020. There are already over a thousand commercial goods on the market that contain nanoparticles (NPs), which have a size range of 1 to 100 nm[1], with numerous wide-ranging applications in the fields of biomedicine, food processing and packaging, agriculture, horticulture, crop protection, wastewater treatment, and environmental remediation[2]. In comparison to their bulk counterparts, NPs have greater chemical reactivity, strength, and some novel properties due to their increased surface-to-volume ratio and quantum size effect. One of the most significant properties of metal NPs is the surface plasmon resonance (SPR) that they show. NPs can be created using the top-down breakdown approach or the bottom-up buildup method[3], involving various physicochemical techniques. However, these production methods are usually expensive, labour-intensive, and potentially hazardous to the environment and living organisms. The bottom-up approach is the most feasible and efficient method for nanoparticle preparation. Biological methods for NP synthesis utilise a bottom-up approach with the help of reducing and stabilising agents[4]. India is rightfully referred to as the "Botanical Garden of the World," as it is the largest producer of medicinal plants in the world. Traditional herbal medicine momentarily became less effective as a result of the advent of modern medicine, but it has since made a comeback, and an "herbal renaissance" is currently blossoming around the world[5]. Plants have been a rich source of medicines because they produce a wide array of bioactive molecules, most of which probably evolved as a chemical defence against predation or infection. The medicinal plants are rich in secondary metabolites and are often termed medicinal or officinal plants. These secondary metabolites or products exert a profound physiological effect on the mammalian system and are thus known as the "active

principles of the plant"[6]. The use of crude drugs of plant origin (unpurified preparations of active principles, plant extracts, or sometimes powdered plant material) is used in the Indian system of medicine, or ayurveda[7]. *Cynodon dactylon* is a perennial grass. The plant is a rich source of metabolites such as proteins, carbohydrates, minerals, and the constituents' flavonoids, alkaloids, glycosides, and triterpenoids. The plant shows biological activities such as antiviral and antimicrobial properties and has been long used in traditional medicines to treat various cancers, cramps, convulsions, coughs, dropsy, dysentery, diarrhoea, headaches, epilepsy, hypertension, anasarca, haemorrhage, hysteria, rubella, measles, sores, snakebite, stones, tumours, urogenital disorders, wounds, and warts. *Cynodon dactylon* belongs to the family Poaceae. It is also known as durva grass, dog's tooth grass, bermuda grass, bahama grass, couch grass, Indian devil's grass, doab, Scotch grass, doob, and durba in different parts of the world. Copper oxide nanoparticles (CuO NPs) are particularly well-known for their biological properties, electric, optical, catalytic, superconducting, and photonic properties[9]. However, their large-scale production has introduced risks to the environment and human health. Considering the wide-ranging applications and increasing demand for metal NPs, an alternative, cost-effective, safe, and green technology for large-scale production of CuO NPs is required. Due to their simplicity and a variety of important physical properties, including high-temperature superconductivity, spin dynamics, and electron correlation effects, CuO NPs have drawn a lot of attention. They are the simplest members of the copper salt family. CuO NPs are utilised to increase the viscosity of energy-transfer fluids, thereby increasing their thermal conductivity, due to their distinctive features and prospective applications. CuO NPs are utilised as heterogeneous catalysts, antioxidants, antibacterial drug delivery agents, and imaging agents in the field of biomedicine.[10] These CuO NPs synthesised using plant extracts may be implemented as chemotherapeutic agents in the near future. In addition to their well-known catalytic action and their antibacterial, antioxidant, and anticancer capabilities, modified CuO NPs have recently been used in medication and gene delivery, which has given them a distinct reputation[11, 12, 13].

MATERIALS AND METHODS

Plant extract preparation

Cynodon dactylon leaf extract was used to synthesise CuO NPs. The leaves of the plant were collected from the university campus (Anna University, Chennai, Tamil Nadu, India), dried, and powdered. About 10g of leaf powder were taken in a 200-ml conical flask, and 100 ml of water were added to the flask (leaves:water = 1:10). Heated at 70–80 °C until the water turned to boil, which in turn led to the solution turning greenish-yellow. The extract was cooled down and filtered using Whatman filter paper.

Preparation of copper oxide nanoparticles

A volume of 50 ml of leaf extract was added to a 50 ml $CuSO_4.5H_2O$ solution and kept overnight at room temperature. The copper salts may undergo bioreduction, turning into copper oxide. The solution was procured and filtered through No. 1 Whatman filter paper. Then the filtrate was dried in a hot air oven at 120°C, and the powdered nanoparticle was collected.

DPPH-radical scavenging activity

DPPH radical scavenging activity was carried out using the method of Molyneux (2004)[14]. To 1 ml of 100 µM DPPH solution in methanol, an equal volume of the test sample in ethanol of different concentrations was added and incubated in the dark for 30 minutes. The change in coloration was observed in terms of absorbance using a spectrophotometer at 514 nm. In place of the test sample, 1 ml of methanol was added to the control tube. Different concentrations of ascorbic acid were used as reference compounds. The percentage of inhibition was calculated from the equation [(Absorbance of Control - Absorbance of Test) / Absorbance of Control] X 100. The GraphPad Prism 5.0 was utilised to compute the IC_{50} value.

Hydrogen Peroxide radical scavenging activity

The hydrogen peroxide radical scavenging activity of the test sample was estimated by the method of Ruch et al. (1989)[15]. A solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4). About 200µl of sample containing different concentrations was mixed with 0.6 ml of H_2O_2 solution. The absorbance of H_2O_2 was determined 10 minutes later against a blank solution containing phosphate buffer without H_2O_2. A test tube containing 200 l of phosphate buffer and processed as described above served as the control tube. Different concentrations of ascorbic acid were used as reference compounds[16].

Characterization of CuO NPs

The synthesised CuO NPs were analysed using SEM, UV-VIS spectra, FTIR spectra, and XRD analysis.

RESULTS AND DISCUSSIONS

DPPH-radical and Hydrogen Peroxide radical scavenging activity

The DPPH radical and H_2O_2 scavenging activity were detected and compared with vitamin C. The activity of DPPH and H_2O_2 radical scavenging in an aqueous extract of *Cynodon dactylon*[16-19] and ascorbic acid were presented in Table 1 and Figures 1a and 1b. The percentage of inhibition in DPPH at different concentrations, like 50, 40, 30, 20, and 10 µg/ml was observed at 81.2, 77.3, 61.3, 42.5, 25.4 and percentage of inhibition in H_2O_2 was observed at 66.18, 60.5, 55.9, 38.4, 27.9 respectively. Whereas, the percentage inhibition of ascorbic acid at different concentrations, like 50, 40, 30, 20, and 10 µg/ml was found to be 91.76, 85.26, 67.70, 51.20, 45.26 and percentage of inhibition in H_2O_2 was observed at 90.11, 82.45, 77.9, 58.92, 42.57 respectively. The IC50 values for DPPH and

H_2O_2 scavenging activity for aqueous extracts of *Cynodon dactylon* and ascorbic acid were 25µg/mL and 19 µg/mL and 18µg/mL and 15 µg/mL, respectively. The higher inhibition activity was recorded in the aqueous extract of *Cynodon dactylon* synthesised CuO NPs in a dose-dependent manner. Values are the average of triplicate experiments and are represented as the mean standard deviation.

Table 1: DPPH radical and H_2O_2 scavenging activity was detected and compared with vitamin C

S.No	Scavenging activity	Concentration (µg/mL)	% of Inhibition DPPH-radical (µg/mL)	IC_{50} values for DPPH	% of Inhibition H_2O_2 radical (µg/mL)	IC_{50} values for H_2O_2
1.	Cynodon dactylon	50	81.2	25 µg/mL	66.18	19µg/mL
		40	77.3		60.5	
		30	61.3		55.9	
		20	42.5		38.4	
		10	25.4		27.9	
2.	Ascorbic acid	50	91.76	18 µg/mL	90.11	15 µg/mL
		40	85.26		82.45	
		30	67.70		77.9	
		20	51.20		58.92	
		10	45.26		42.57	

1a DPPH radical scavenging activity of aqueous extract of *Cynodon dactylon*

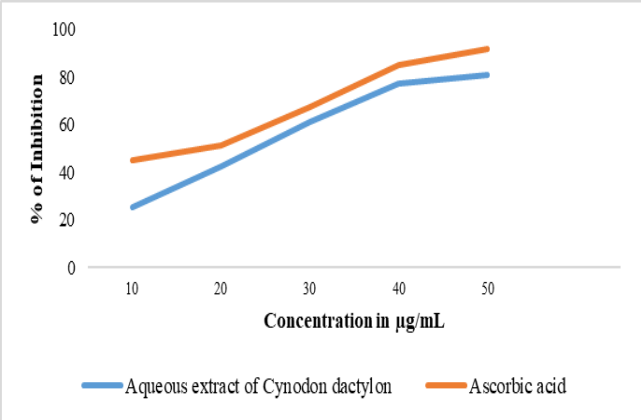


Figure 1a: The DPPH radical scavenging activity of Cynodon dactylon was detected and compared with vitamin C.

1b H_2O_2 radical scavenging activity of aqueous extract of *Cynodon dactylon*

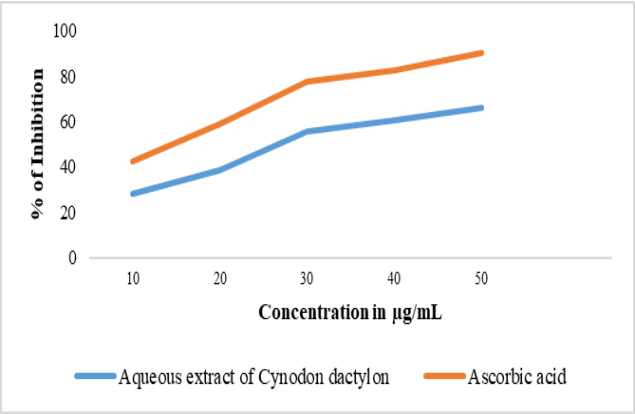
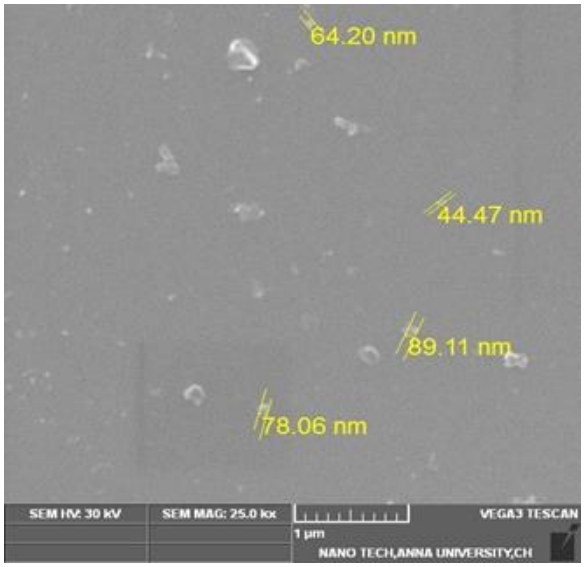
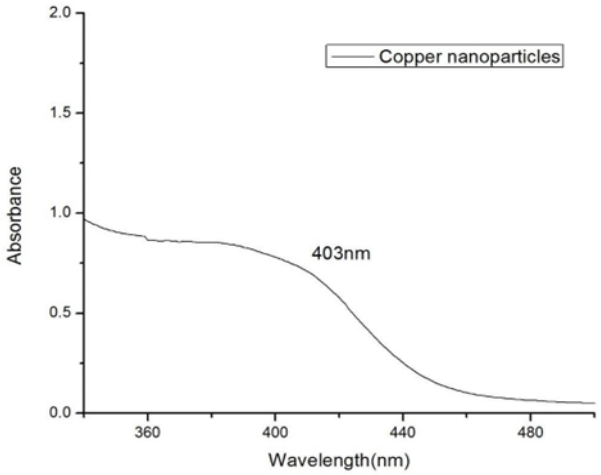


Figure 1b: The H_2O_2 radical scavenging activity of Cynodon dactylon was detected and compared with vitamin C.

Characterization of CuO NPs

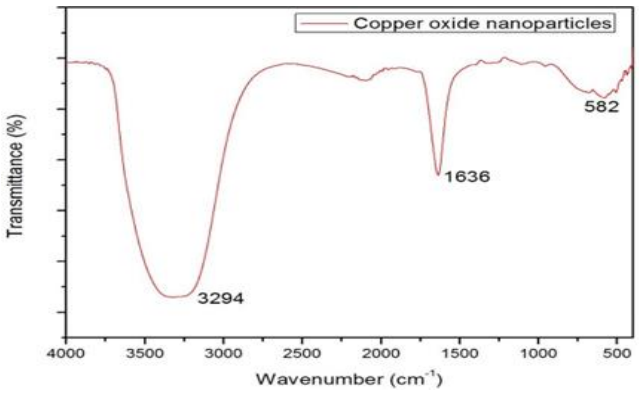


2a SEM analysis of CuO NPs

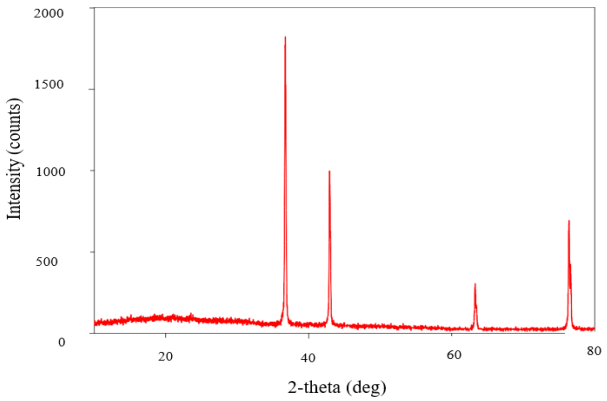


2b UV-VIS SPECTRA analysis of CuO NPs

Figures 2a and 2b. The SEM and UV-visible absorption spectrum images of CuO NPs as prepared from copper sulphate are shown. In figure 2a, it clearly shows the well-dispersed, spherical-shaped distribution of CuO NPs prepared with *Cynodon dactylon* extract, with particle sizes ranging under 70 nm. In figure 2b, the copper nanoparticles prepared have displayed an absorption peak at between 390 and 410 nm, which is assigned to the absorption of CuO NPs. This spectrum confirms the presence of CuO only, as there is no other measurable peak observed. The incidence of the surface plasmon absorption reflects the shape and size of the nanoparticles.



3a FTIR SPECTRA analysis of CuO NPs



3b XRD analysis of CuO NPs

Figure 3a & 3b: The broad and strong peak at around 3294 cm1 can be attributed to the synthesized CuO NPs. In Figure 3a the band at 1636 cm^{-1} is assigned to C-H stretching. The major peak was observed to be 582 cm^{-1} should be a stretching of Cu-O. Figure 3b depicts the XRD pattern of CuO nanoparticles. The sharp diffraction peaks in the XRD pattern distinctly depict the crystalline nature of the sample. According to the standard JCPDS data card, the standard diffraction peaks representing the crystal structure of CuO are hexagonal wurtzite. Diffraction peaks of other impurities were not detected. This proved that the peaks that were observed in the XRD spectrum belonged only to the Cu.

The green synthesis of copper oxide nanoparticles was successfully done using the leaf extract of *Cynodon dactylon*. Identification and characterization of the synthesised CuO NPs by UV-vis spectroscopy is a very useful technique for analysing nanoparticle formation and the stability of metal nanoparticles in aqueous solutions[20-23]. The DPPH radical scavenging activity and the H_2O_2 radical scavenging activity were detected and compared with vitamin C. The activity of DPPH radical scavenging and H_2O_2 radical scavenging in an aqueous extract of *Cynodon dactylon* and ascorbic acid shows more antioxidant properties of CuO NPs, suggesting that *Cynodon dactylon* may be used as an application of antioxidants that exist naturally to prevent diseases. The characteristics of the obtained CuO nanoparticles were studied using UV-vis and FTIR techniques. Absorption peaks at between 390 and 410 nm, which is assigned to the absorption of CuO NPs. Based on the peak value in the region of infrared radiation, FTIR spectroscopy was employed to determine the functional groups of the active components. The broad and strong peak at around 82cm^{-1} can be attributed to the synthesised CuO NPs. The UV-vis spectrum and FTIR spectrum confirm the synthesised copper oxide nanoparticles.

CONCLUSION

The synthesised CuO NPs are highly stable and have a significant effect on antioxidant properties. This is a low-cost synthesis method, and it does not require any toxic chemicals for the synthesis. It is summarised that the present method of synthesis of CuO nanoparticles provides strong potential for the future development of green nanomaterials. The green synthesis method has many advantages, such as economic viability, ease of scaling up, and being less time-consuming. For the synthesis of nanoparticles, using ecologically friendly ingredients like plant extracts has many advantages in terms of compatibility and friendliness with the environment.

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REFERENCES

1. Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella Jr MF, Rejeski D, Hull MS. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. Beilstein journal of nanotechnology. 2015 Aug 21;6(1):1769-80.
2. Siddiqi KS, Husen A. Recent advances in plant-mediated engineered gold nanoparticles and their application in biological system. Journal of Trace Elements in Medicine and Biology. 2017 Mar 1;40:10-23.
3. Husen A, Siddiqi KS. Phytosynthesis of nanoparticles: concept, controversy and application. Nanoscale research letters. 2014 Dec;9:1-24..
4. Joshi A, Sharma A, Bachheti RK, Husen A, Mishra VK. Plant-mediated synthesis of copper oxide nanoparticles and their biological applications. Nanomaterials and Plant Potential. 2019:221-37.
5. Sumathi P, Parvathi A. Antimicrobial activity of some traditional medicinal plants. Journal of Medicinal plants research. 2010 Feb 18;4(4):316-21.

6. Chothani DL, Vaghasiya HU. A review on Balanites aegyptiaca Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity. Pharmacognosy reviews. 2011 Jan;5(9):55.
7. Suri SS, Arora DK, Ramawat KG. A method for large-scale multiplication of Curculigo orchioides through bulbil formation from leaf explant in shake flask culture.
8. Nagori BP, Solanki R. Role of medicinal plants in wound healing. Research Journal of Medicinal Plant. 2011;5(4):392-405.
9. Padil VV, Černík M. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. International journal of nanomedicine. 2013 Feb 28:889-98.
10. Ijaz F, Shahid S, Khan SA, Ahmad W, Zaman S. Green synthesis of copper oxide nanoparticles using Abutilon indicum leaf extract: Antimicrobial, antioxidant and photocatalytic dye degradation activities. Tropical Journal of Pharmaceutical Research. 2017 May 4;16(4):743-53.
11. Vishveshvar K, Aravind Krishnan MV, Haribabu K, Vishnuprasad S. Green synthesis of copper oxide nanoparticles using Ixiro coccinea plant leaves and its characterization. BioNanoScience. 2018 Jun;8:554-8.
12. Padil VV, Černík M. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. International journal of nanomedicine. 2013 Feb 28:889-98.
13. Rehana D, Mahendiran D, Kumar RS, Rahiman AK. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. Biomedicine & Pharmacotherapy. 2017 May 1;89:1067-77.
14. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. sci. technol. 2004 Dec;26(2):211-9.
15. Ruch RJ, Crist KA, Klaunig JE. Effects of culture duration on hydrogen peroxide-induced hepatocyte toxicity. Toxicology and applied pharmacology. 1989 Sep 15;100(3):451-64.
16. Heer AS. Spectral characterization and anti-fungal activity of zinc oxide (zno) nanoparticles synthesized using *Cynodon dactylon* leaf extract. World Journal of Pharmaceutical Research. 2017 Oct 16;6:16.
17. Altikatoglu M, Attar A, Erci F, Cristache CM, Isildak I. Green synthesis of copper oxide nanoparticles using Ocimum basilicum extract and their antibacterial activity. Fresenius Environ. Bull. 2017 Jan 1;25(12):7832-7.
18. Sivaraj R, Rahman PK, Rajiv P, Narendhran S, Venckatesh R. Biosynthesis and characterization of Acalypha indica mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014 Aug 14;129:255-8.
19. Sankar R, Maheswari R, Karthik S, Shivashangari KS, Ravikumar V. Anticancer activity of Ficus religiosa engineered copper oxide nanoparticles. Materials Science and Engineering: C. 2014 Nov 1;44:234-9.
20. Bachheti RK, Fikadu A, Bachheti A, Husen A. Biogenic fabrication of nanomaterials from flower-based chemical compounds, characterization and their various applications: a review. Saudi Journal of Biological Sciences. 2020 Oct 1;27(10):2551-62.
21. Sampath M, Vijayan R, Tamilarasu E, Tamilselvan A, Sengottuvelan B. Green synthesis of novel jasmine bud-shaped copper nanoparticles. Journal of Nanotechnology. 2014 Jan 1;2014.
22. Kaur R, Giordano C, Gradzielski M, Mehta SK. Synthesis of highly stable, water-dispersible copper nanoparticles as catalysts for nitrobenzene reduction. Chemistry–An Asian Journal. 2014 Jan;9(1):189-98.
23. Din MI, Arshad F, Hussain Z, Mukhtar M. Green adeptness in the synthesis and stabilization of copper nanoparticles: catalytic, antibacterial, cytotoxicity, and antioxidant activities. Nanoscale research letters. 2017 Dec;12:1-5.

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