Green Synthesis of Copper Oxide Nanoparticles from Callus Culture of *Echinacea purpurea* and their Biomedical Potential

By

Bushra Khan

Registration no: 02272113014

Department of Biotechnology
Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad, Pakistan

2023

Green Synthesis of Copper Oxide Nanoparticles from Callus Culture of *Echinacea purpurea* and Their Biomedical Potential

By

Bushra Khan

Supervised by
Dr. Bilal Haider Abbasi

A thesis submitted in the partial fulfillment of the requirements for the degree of

MASTER OF PHILOSOPHY

In

Biotechnology

Department of Biotechnology
Faculty of Biological Sciences,
Quaid-I-Azam University Islamabad,
Pakistan
2023


بِسْمِ ٱللَّهِ ٱلرَّحْمَـٰنِ ٱلرَّحِيمِ
IN THE NAME OF ALLAH

AUTHOR'S DECLARATION

I, BSHRA KHAN, daughter of ARSHAD MEHMOOD KHAN, bearing Registration No. 02272113014 and enrolled as an MPhil Biotechnology scholar in the Department of Biotechnology, Faculty of Biological Sciences at Quaid-i-Azam University Islamabad, Pakistan, hereby affirm that the information cited in the thesis titled "Green synthesis of copper oxide nanoparticles from callus cultures of *Echinacea purpurea* and their biomedical potential" is derived from original research conducted under the guidance of Dr. Bilal Haider Abbasi. This work has not been submitted or published elsewhere.

Furthermore, I also understand the terms 'copyright' and 'plagiarism'. If evidence of plagiarism is found at any level the university will have the right to revoke or cancel my degree even after the degree award. Moreover, the plagiarism of this thesis has been checked via Turnitin software.

Signature: _____

Bushra Khan

Dated: _____

DEDICATION

I dedicate my thesis work to my incredible parents ***(Mr. and Mrs. Arshad Mehmood Khan)*** *and my beloved siblings* ***(Rubab Arshad, Hira Khan, Mahnoor Khan and Muhammad Umer Khan)****. Their boundless love, unwavering support, and endless encouragement have been the driving forces behind my academic journey. Their sacrifices have paved the way for my accomplishments, and their constant presence has provided the inspiration to persevere. This work is a tribute to their faith in my abilities and a reflection of the values they have instilled in me. Thanks to them for being the foundation of my achievements and for showing me the true meaning of dedication and determination.*

BUSHRA KHAN

ACKNOWLEDGEMENTS

All gratitude is directed towards the **Almighty Allah**, the all-powerful and merciful, as well as His **Prophet Muhammad** ﷺ, who stands as the epitome of perfection among all individuals to have graced the Earth. His teachings and wisdom continue to serve as an eternal beacon of guidance and knowledge for the entirety of humanity.

I express my sincere gratitude to my supervisor and mentor, **Professor Dr. Bilal Haider Abbasi**, a Tenured Professor at the Department of Biotechnology at QAU, Islamabad, Pakistan. I am profoundly thankful for the exceptional guidance and mentorship that he has offered me during the course of my thesis research. His knowledge, persistence, and astute criticism played a crucial role in determining the scope and calibre of this study. His commitment to helping me succeed academically has motivated me to go beyond my strength and set high standards for myself.

I would also like to extend my heartfelt appreciation to my dearest friend **Syed Muhammad Hussain Arif Billah**. His unwavering support, countless brainstorming sessions, and constructive discussions have truly been a driving force behind the completion of this thesis. His willingness to lend an ear during moments of frustration and to celebrate every milestone reached has made this journey memorable and rewarding. I also cannot forget to pay my deepest gratitude to my dearest friends and lab fellows **Tehreem Mahmood, Uzair Javaid and Gouhar Zaman** for their continuous professional advice and help that enabled thought-provoking ideas for me during the research work. Their collective contributions have not only enriched the content of this work but have also made the entire process more enjoyable and fulfilling. I want to express my sincere gratitude to everyone who assisted me in any manner when I was staying at the Department of Biotechnology, QAU Islamabad, Pakistan. I collaborated with a lot of people throughout my M.Phil. study, and I want to express my appreciation to each and every one of them for their special spirit of generosity.

Thank you for believing in me, for your unending encouragement, and for being an integral part of this significant accomplishment in my academic endeavors. May Allah bless you all with eternal happiness and success! Ameen.

(Bushra Khan)

Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree centigrade
$CuSO_4.5H_2O$	Copper sulphate
CuO-NPs	Copper oxide nanoparticles
DMSO	Dimethyl sulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DW	Dry weight
E. purpurea	*Echinacea purpurea*
FC	Folin-Ciocalteu
fDDA	Fast data dependent mode
FRAP	Free radical scavenging activity
FW	Fresh weight
L/D	Light/Dark
MIC	Minimum Inhibitory Concentration
MS	Murashige and Skoog
MS	Mass spectrometry
NAA	α-Naphthalene acetic acid
QE	Quercetin
ROS	Reactive oxygen species
SD	Standard deviation
TFC	Total Flavonoid Content
TPC	Total Phenolics Content
TEM	Transfer Electron Microscopy

TEAC	Trolox Equivalent Antioxidant Capacity
g /L DW	Gram per liter dry weight
g /L FW	Gram per liter fresh weight
µg/mg	Microgram per milligram
µM	Micromolar
mg/L	Milligram per liter
min	Minutes

Abstract

Rapid strides in various industries have propelled the swift evolution of nanotechnology. The last few years have witnessed remarkable progress in research involving nanomaterials, particularly within the realm of nanotechnology. Among the spectrum of nanoparticles, copper oxide nanoparticles (CuO-NPs) have emerged as a focal point, given their diverse properties and wide-ranging applications across multiple domains. Various methodologies exist for synthesizing copper oxide nanoparticles, with chemical, physical, and biological approaches being prominent. However, the physicochemical techniques prove to be not only costly but also environmentally hazardous due to the high energy consumption and release of toxic chemicals. Conversely, the biological method presents itself as an eco-friendly, cost-efficient, dependable, user-friendly, and uncomplicated route for producing copper oxide nanoparticles. This study reports the green-mediated synthesis of CuO-NPs using callus extract of *Echinacea purpurea* and their biomedical applications. The synthesized copper oxide nanoparticles (CuO-NPs) undergo characterization using a range of techniques including UV/VIS spectrophotometry, Fourier-transformed infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), and dynamic light scattering (DLS). UV/VIS spectrophotometry showed the highest peak at 284nm which confirmed the synthesis of CuO-NPs. The cubic character of the nanoparticles, with an average size of 32.58nm, was confirmed by the XRD pattern. Utilizing FTIR analysis, the functional groups accountable for both capping and size reduction of the copper oxide nanoparticles were investigated. Notably, distinctive peaks corresponding to CuO nanoparticles were identified at approximately 593 cm^{-1}. Through the application of SEM, it was possible to determine that green-mediated CuO-NPs were 40–80 nm-sized, spherical in nature, evenly dispersed, and free of aggregation. EDS analysis indicated the presence of oxygen and copper by 36.16% and 35.42% corresponding to the peaks recorded at 1 keV and 8 keV. The DLS investigation revealed that the zeta value of biosynthesized CuO-NPs from *Echinacea purpurea* is 6.78 mV, demonstrating the relative stability of synthesized nanomaterials. The synthesized CuO-NPs were also analyzed for their phytochemical profile and antioxidant potential. The maximum values of TPC (24.96 µg/mg) and TFC (69.85 µg/mg) were recorded against the highest concentration of CuO-NPs (80 µg/mL). The optimal values for DPPH (95.45%) and TAC (18.48 µg AAE/mg DW) were also reported against the highest concentration of CuO-NPs. The results of all these activities were observed to be dosage dependent i.e., the values of the TPC, TFC, TAC

and DPPH increased with increasing concentration of CuO-NPs. Our research showcases the uncomplicated and environmentally friendly synthesis of CuO nanoparticles (CuO-NPs), highlighting their noteworthy potential in the realm of biomedicine. Subsequent investigations hold the promise of unveiling the therapeutic capacities of CuO-NPs, necessitating in-depth explorations involving both in vivo and in silico analyses.

CHAPTER 1

INTRODUCTION

1. Introduction:

The inception of nanotechnology was initially introduced by the Nobel laureate in physics, Richard P. Feynman, during his renowned presentation titled "There is Plenty of Room at the Bottom." This seminal event occurred at the American Physical Society gathering in December 1959 (Benelmekki, 2015). Since then, numerous breakthrough advances in the realm of nanotechnology have occurred. Numerous nanoscale materials have been produced due to nanotechnology. A large category of materials known as "nanoparticles" (NPs) includes particulate compounds with at least one dimension less than 100 nm (Laurent et al., 2008). When researchers discovered that a the size and shape of a substance might impact its physiochemical characteristics, such as optical qualities, the significance of these materials became apparent (I. Khan et al., 2019). Top-down and bottom-up are the two approaches to the nanoscale. The top-down approach involves the reduction of the structure to the nanoscale, while the bottom-up approach revolves around constructing a larger nanostructure from smaller atoms and molecules (Christian et al., 2008). NPs are classified into several types based on their morphology, size, and physical and chemical properties, such as carbon-based NPs, metal NPs, ceramic NPs, semiconductor NPs, polymeric NPs, and lipid-based NPs (Bommakanti et al., 2022). NPs have an extensive array of applications in numerous spheres of life due to their unique features. They are employed as drug delivery carriers in healthcare systems and are rapidly being used in wound dressings, MRI, pharmaceutical medicines, and diagnostics (AshaRani et al., 2009). NPs are also being utilized more frequently in the material sector to make microelectronics, aerospace products, food processing and packaging, bio-photonics, and medicinal items (Lei et al., 2015). Environmental uses for NPs include wastewater treatment, bioremediation, the production of environmentally sustainable products, biosensors, and nanosorbents (Mueller & Nowack, 2008). Nanoparticles (NPs) find wide-ranging applications in diverse energy harvesting technologies. These include electrochemical CO2 reduction for fuel precursor production, photoelectrochemical (PEC) and electrochemical water splitting, solar cells, piezoelectric devices, and nanogenerators (Song et al., 2016).

Metal oxide nanoparticles have attracted considerable attention owing to their wide-ranging applications across various industries. They serve as indispensable components in industrial catalysts, chemical sensors, medical applications, disinfection and antimicrobial agents, fillers, opacifiers, catalysts, semiconductors, and contribute significantly to the advancement in

cosmetics and microelectronics (Katwal et al., 2015). Due to its antibacterial and biocidal properties, copper oxide (CuO) has grown in popularity and has the potential to be utilized in a range of biomedical domains (Nations et al., 2015). Copper oxide is a semiconductor metal with unique optical, electrical, and magnetic properties that has been employed in the development of supercapacitors, near-infrared filters, magnetic storage media, sensors, catalysis, semiconductors, and other applications (Dagher et al., 2014). CuO-NPs have been recognized for their antibacterial properties, making them effective agents in hospitals for eradicating over 99.9% of bacteria within a mere 2 hours of exposure, provided the appropriate dosage is administered (Grigore et al., 2016). CuO-NPs have been demonstrated to have favorable skin effects in prior studies. Studies on women who used bedding and pillowcases treated with copper oxide nanoparticles (CuO-NPs) revealed improved skin on the face and more flexible foot skin when socks were worn (Dykes, 2015). The capacity of these nanoparticles to heal wounds is another possible application. There are numerous wound dressings and materials available to treat burns and other skin ailments. It has been demonstrated that there is a direct correlation between the capacity of CuO-NPs to restrict microbial colonization of treated areas, prevent infection, and promote the regeneration of wounded tissue (Thampi et al., 2015).

CuO-NPs are synthesized using a variety of physical and chemical procedures such as co-precipitation, microemulsion, ultrasound, hydrothermal synthesis, microwave, spark discharge, inert gas condensation, laser ablation, sputtering, sol-gel, and so on (Grigore et al., 2016) . These processes have several drawbacks, including high costs, radiation exposure, a high energy need, high temperature and pressure, low purity, the use of hazardous chemicals and organic solvents, and a substantial volume of waste creation (Bloch et al., 2021). Given these drawbacks, scientists have chosen biological pathways as a potential technique of producing CuO nanoparticles since they are cost-effective, biocompatible, and environmentally benign. Metals and metal oxide nanoparticles have recently been produced by plants, bacteria, fungi, and algae. The shape and appearance of generated nanoparticles are significantly influenced by the nature of biological entities. The wide range of biological entities has resulted in an intriguing assortment of nanoparticle shapes and sizes, with the entities serving as a template for nanoparticle creation (Pandit et al., 2022).

The notion of "Green Chemistry", over the past ten years, for sustainable development has drawn a lot of attention. The three most significant prerequisites for green NP synthesis are the use of a green or ecologically friendly solvent (the most commonly used are water, ethanol, and their combinations), a suitable non-toxic reducing agent, and a safe chemical for stabilization (Omer, 2008). Many different NPs have been synthesized using plant materials like fruits, leaves, seeds roots and stems (Narayanan & Sakthivel, 2011) . Plant extracts can, in fact, synthesize NPs with certain sizes, shapes, and compositions. Additionally, a range of phytochemicals present in their extract may work inadvertently to stabilize and/or reduce the synthesis of NPs (Hano & Abbasi, 2021). With applications in agriculture, food science and technology, bioengineering, cosmetics or nanomedicine, and human health protection, plant derived CuO-NPs have a strong biological propensity and are less likely to have serious adverse effects on humans than chemically manufactured counterparts (Razavi et al., 2015).

Plants have been frequently exploited in the synthesis of CuO-NPs due to their low cost, non-toxicity, considerable bioactive substances, ease of availability, and environmental friendliness. The production of CuO-NPs using aromatic and medicinal herbs revealed a wide range of biological activities including antioxidant, antiviral, anticancer, antifungal, anti-inflammatory and antibacterial properties (Beyene et al., 2017). There are 11 species in the genus *Echinacea*, which is an aromatic and medicinal plant, a part of the Asteraceae family and native to North America. (Thomsen et al., 2018). Many diseases, including snake bites, wound infections, colds, coughs, bronchitis, and immunodeficiency diseases have been treated with the use of the genus species in herbal medicine (Tsai et al., 2012). *Echinacea pallida, Echinacea angustifolia and Echinacea purpurea* are three economically important Echinacea species used to treat bacterial and viral diseases (Barrett, 2003). It has been demonstrated that *Echinacea purpurea* (L.) Moench possesses a variety of biological properties, including anti-cancer (Erenler et al., 2015), antioxidant (Tsai et al., 2012), antibacterial, antidiabetic (Carvalho et al., 2016), and antifungal (Liu et al., 2015). Polysaccharides, alkaloids, alkyl amides, and polyphenols have all been discovered as a result of phytochemical studies on the plant *Echinacea purpurea* (Catanzaro et al., 2018). *Echinacea purpurea* has been utilized as an herbal treatment in many cultures. Preclinical research was conducted, and *Echinacea* extracts shown antibacterial efficacy

against microorganisms accountable for respiratory illnesses. Furthermore, skin infections could be a potential target (Sharifi-Rad et al., 2018).

This study is focused on synthesizing copper oxide nanoparticles using callus cultures of *Echinacea purpurea.* The callus culture was obtained using TDZ as a plant growth regulator. UV/VIS spectrophotometry, Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy, Energy Dispersive X-ray Spectroscopy (EDS), X-Ray Diffraction Analysis and Dynamic Light Scattering (DLS) were used to characterize CuO nanoparticles. These analyses were done to confirm the particle size, morphology and presence of functional groups that take part in the capping and reduction of the copper ions. FTIR depicts the adsorption bands of the functional groups attached to nanoparticles. XRD is used to confirm the elemental composition and crystallinity of nanoparticles. SEM is done to analyze the morphological features of nanoparticles. DLS is performed to analyze surface charge and particle size of nanoparticles. To study the biomedical potential of *Echinacea purpurea* callus based CuO nanoparticles, various assays were performed such as an antioxidant assay, anti-bacterial assay, total phenolic content, total flavonoid content and total antioxidant capacity that helped in analyzing the true potential of CuO nanoparticles as reducing, stabilizing, capping, antioxidant and anti-microbial agent.

1.1 Aims

Metal-based nanoparticles have received a significant focus in the biomedical field. In addition to their smaller size and bacterial selectivity, metal-based nanoparticles have been demonstrated to be effective against infections. In addition to making bacterial resistance difficult to develop, metal-based nanoparticles have non-specific bacterial toxicity mechanisms (they do not connect to a specific receptor in the bacterial cell). This broadens the antibacterial effectiveness spectrum. Green synthesis has proven to be an effective method for mitigating the potential adverse effects of chemically and physically generated metal nanoparticles. The aim of the research is to biosynthesize copper oxide nanoparticles utilizing *Echinacea purpurea* callus culture. The callus culture is produced using stem and leaf explants of *Echinacea purpurea* while utilizing TDZ as a plant growth regulator. The produced NPs will be characterized using a variety of techniques including FTIR, XRD, UV/VIS spectrophotometry, DLS, and SEM. A comparison of the antioxidant and antibacterial capability of CuO-NPs and *Echinacea purpurea* plant extract is also carried out using several assays such as DPPH activity, TAC, and anti-bacterial activity via agar

well diffusion. To quantify and compare the levels of flavonoids and phenols in both samples, the plant extract and copper oxide nanoparticles are subjected to Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) assays.

1.2 Objectives

This study has following objectives:-

- Production of callus cultures of *Echinacea purpurea* from stem and leaf explants using TDZ as a plant growth regulator.
- Synthesis of copper oxide nanoparticles through the green approach using *Echinacea purpurea* callus.
- Characterization of *Echinacea purpurea* callus-based biosynthesized copper oxide nanoparticles using UV/VIS spectrophotometer, XRD, DLS AND SEM to understand the size and morphology and FTIR to study the attached functional groups.
- To study the TPC and TFC of green-mediated copper oxide nanoparticles.
- To examine the total antioxidant capacity (TAC) and antioxidant capability of copper oxide nanoparticles synthesized through a biosynthetic process using the DPPH assay.

CHAPTER 2

LITERATURE REVIEW

2. Literature Review:

2.1 Nanotechnology and Nanobiotechnology

Nanotechnology is concerned with the manipulation, investigation, and development of materials and technologies at the nanoscale scale, which typically ranges from one to a few hundred nanometers (Thakkar et al., 2010). Matter exhibits distinct and frequently amazing properties at this microscopic dimension that differ from their bulk counterparts. This opens up a world of possibilities for designing and engineering materials with extraordinary capabilities, changing industries ranging from electronics and health to energy and manufacturing (Zharov et al., 2005).

The cutting-edge interdisciplinary field of nanobiotechnology combines the complex realm of biology with the principles of nanotechnology. The idea of biogenic reduction by nanoparticles is an intriguing feature of nanobiotechnology. The reduction of metal ions into their elemental forms inside biological systems is catalyzed and facilitated by the usage of nanoparticles, which are typically made of metal or metal oxide (Vaseghi et al., 2018). Targeted drug administration, imaging, and biosensing are just a few of the novel biomedical applications that can now be applied by scientists by taking advantage of the special features of nanoparticles. These reduction reactions may now be manipulated and controlled with high efficiency and precision. Furthermore, nanobiotechnology uses a bottom-up strategy in which nanoscale components are put together and arranged starting at the molecular level to create sophisticated structures and systems (S. C. Singh et al., 2010). Scientists may manage and control reduction reactions with great accuracy and efficiency by utilising the special features of nanoparticles, creating new opportunities in many biological applications, including targeted drug delivery, imaging, and biosensing. Additionally, nanobiotechnology uses a bottom-up strategy in which molecularly level nanoscale components are integrated and organised to create complex structures and systems (Bayda et al., 2019).

2.2 Nanoparticles

Nanoparticles are microscopic objects with a size between one and one hundred nanometers and can be made of carbon, metal, metal oxides, or organic material (Hasan, 2015). Nanoparticles differ physically, chemically, and biologically from their corresponding particles at larger scales. Increased chemical reactivity or stability, increased mechanical strength, etc. are all factors

contributing to this impact, as well as a surface area to volume ratio that is much higher than average (P. Biswas & Wu, 2005). Nanoparticles are used in many different applications as a result of their unique features. Long before their origins and characteristics were discovered and understood, nanoparticles were used in stained glass, paints, and building materials. Transition metal nanoparticles have been utilised as heterogeneous catalysts for more than a century, and they have produced significant profits for the petrochemical industry (Heiligtag & Niederberger, 2013).

Since NPs are complex molecules, they contain three layers: (a) the surface layer, which may be functionalized with a range of small molecules, metal ions, surfactants, and polymers. The core, which often refers to the NP itself, is the central region of the nanoparticle (Shin et al., 2016). The shell layer and the core are chemically separate from one another in all respects (Shin et al., 2016). The exceptional qualities of these materials have aroused the interest of scientists from several fields.

2.3 Classification of Nanoparticles

2.3.1 Based on Origin

Depending on from the source they origin, nanomaterials can be divided into two groups: natural nanoparticles and synthetic nanoparticles.

2.3.1.1 Natural nanomaterials

Nature presents a diverse array of natural nanomaterials, encompassing a diversity of natural forms including protein molecules, viruses, liquid colloids like milk and blood, gelatin that takes on a gel-like state, mineralized substances like spider silk, insect wings as well as structures like shells, corals, and bones. Additionally, materials such as lotus leaves, gecko feet, volcanic ash, and ocean spray contribute to this rich spectrum of nanoscale entities (Cho et al., 2019).

2.3.1.2 Artificial nanomaterials

Quantum dots (QDs), a type of semiconductor nanoparticle, and carbon nanotubes are two examples of man-made nanomaterials that are created intentionally utilising exact mechanical and manufacturing techniques. Depending on their structural makeup, nanomaterials are divided into three categories: dendrimers, metal-based materials, and composites (M. C. Biswas et al., 2022).

2.3.2 **Based on structural composition/configuration**

Nanoparticles can be generically categorized into four types based on their structural makeup: organic/dendrimers, inorganic, carbon-based, and composites.

2.3.2.1 **Organic Nanoparticles**

Organic molecules are transformed into organic nanomaterials at the nanoscale (I. Ijaz et al., 2020). Liposomes, dendrimers, micelles, and ferritin are a few examples of organic nanoparticles or polymers. Non-toxic, biodegradable nanoparticles with hollow interiors called nanocapsule micelles and liposomes are sensitive to light, heat and electromagnetic radiation (Y. Khan et al., 2022). Dendrimers have multiple chain endings on their surface that can carry out particular chemical reactions. In molecular recognition, nano sensing, light harvesting, and opto-electrochemical systems, dendrimers are employed. Additionally, three-dimensional (3D) dendrimers may be helpful for drug administration since they have interior holes that can contain additional molecules (Mekuye & Abera, 2023).

2.3.2.2 **Inorganic Nanoparticles**

Inorganic nanoparticles are those that do not contain carbon atoms. Typically, metals, metal oxides, semiconductors (diluted magnetic, concentrated magnetic, and non-magnetic), ceramics, and lipid-based nanoparticles make up inorganic nanoparticles (Sannino, 2021).

2.3.2.3 **Carbon-based Nanoparticles**

The five primary components that make up carbon-based nanomaterials are carbon nanotubes, graphene, fullerenes, carbon nanofiber, and carbon black. Bucky balls are fullerenes composed of carbon nanomaterials that have spherical and elliptical shapes (Kristianto et al., 2022). Fullerenes are spherical compounds with diameters of up to 8.2 nm for single layers and 4 to 36 nm for multilayered fullerenes. They are composed of 28 to 1500 carbon atoms. Carbon-based nanoparticles find prevalent application in enhancing structural integrity, often surpassing the strength of steel in certain instances. These nanomaterials, primarily composed of carbon, exhibit the unique property of conducting heat along their length while restricting heat transmission across their structure (Zhang et al., 2021).

2.3.2.4 **Composite Nanoparticles**

Composites Nanoparticles are mixed with other nanoparticles, with materials at a larger size, and with bulk-type materials to form nanomaterials (Gu et al., 2022). Nanomaterials are currently

integrated into numerous products, enhancing attributes like mechanical strength, thermal characteristics, and flame resistance. These applications span diverse industries, encompassing automotive components to packaging materials.

2.3.3 Based on Dimension

According to their size dimensions, nanomaterials can be divided into four categories: zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) (3D). Nanomaterials must be inside the nanoscale range or smaller than 10 nm in each of the three dimensions namely x, y and z to qualify as zero-dimensional materials. QDs and fullerenes are two examples of 0D nanomaterials (F\ind\ik, 2021). ID nanomaterials have two dimensions (x, y) that are contained inside the nanoscale; however, one of the three dimensions of the nanostructure is larger than 10 nm. Nanohorns, nanotubes, nanorods, nanofibers, thin films, and nanowires are prime illustrations of one-dimensional (1D) nanomaterials, characterized by their unique needle-like structure (Afolalu et al., 2019). 2D nanomaterials feature plate-like geometries and two dimensions that fall into the sub-nanometer scale (between 1 and 100 nm), in contrast to 1D nanomaterials, which have one dimension in the nanoscale range. Examples of 2D nanomaterials include thin-film multilayers and coatings, nanosheets or nanowalls etc. (Joudeh & Linke, 2022). The term 3D nanomaterials, often known as bulk materials, refers to nanoparticles that are larger than the nanoscale in any dimension or range of dimensions. While 3D nanomaterials have all three dimensions exceeding 100 nm and are not restricted to the nanometer range or smaller, bulk materials are made up of distinct blocks that are between 1 and 100 nm in size (F\ind\ik, 2021). The intimate coexistence of 0D, 1D, and 2D structural features results in nanoparticle dispersion, interfaces in multi-nanolayers, and aggregates of nanowires and nanotubes. 3D nanomaterials, in contrast, include colloids, free nanoparticles with various morphologies, and thin films with atomic-scale porosity (Lu & Ozcan, 2015).

2.4 Properties of Nanoparticles

Nanomaterials exhibit significant variations in their magnetic, optical, electrical, mechanical, chemical, and physical properties when compared to atoms and bulk materials. These variations are brought about by things like composition, crystallography, surface charge and interaction, surface area, and the impacts of nanoscale size (Rai & Nguyen, 2021). The chemical or elemental makeup of the nanoparticle affects its purity and functionality. The most significant

physicochemical characteristics that are altering at the nanoscale are outlined in the sections that follow.

2.4.1 Mechanical Properties

Mechanical properties pertain to the distinctive responses exhibited by materials under diverse conditions, environmental influences, and external forces. In the realm of nanomaterials, these properties generally encompass ten essential aspects: strength, brittleness, hardness, toughness, fatigue strength, plasticity, elasticity, ductility, rigidity, and yield stress (Q. Wu et al., 2020). Most inorganic, non-metallic materials lack significant toughness, plasticity, elasticity, or ductility attributes, rendering them brittle. On the other hand, organic substances tend to possess flexibility and may not consistently demonstrate rigidity and fragility. Nanoparticles (NPs) showcase unique mechanical characteristics distinct from those of bulk materials, primarily attributed to surface and quantum effects(Guo et al., 2013). Multiple theories have been proposed to elucidate how forces of interaction between nanoparticles (NPs) give rise to unique mechanical properties. For instance, the DLVO hypothesis combines electrostatic repulsion with van der Waals attraction to explain the stability of colloidal dispersions (Missana & Adell, 2000).

2.4.2 Thermal Properties

Energy conduction from electrons and photons (lattice vibration) as well as the scattering processes that follow both are the main drivers of heat transfer in NPs (Savage & Rao, 2004). Thermoelectric power, thermal stability, heat capacity and thermal conductivity are all important aspects to consider the thermal qualities of a material. Nanoparticles (NPs) exhibit a direct influence of their size on both electrical and thermal conductivity. The surface area to volume ratio of NPs grows hyperbolically as their size falls (Andrievski, 2014). Heat is transferred primarily through two main mechanisms, one of which involves the conduction of electrons. Compared to bulk materials, nanoparticles (NPs) with higher surface-to-volume ratios have an abundance of electrons that are available for heat transmission (Qiu et al., 2020). Additionally, microconvection, which results from NPs' Brownian motion, enhances heat conductivity in NPs (Shima et al., 2009). However, this phenomenon only manifests when liquid is used to scatter solid NPs (generating a Nanofluid) (Syam Sundar & Sharma, 2008). For instance, the thermal conductivity of ethylene glycol can be increased by up to 40% by the addition of Cu NPs (Eastman et al., 2001).

2.4.3 Magnetic Properties

In the formula of every magnetic compound (at ambient temperatures), there exists a "magnetic element" like Fe, Co, or Ni. Conversely, diamagnetic elements such as Pd, Au, and Ag are also present. However, on the nanoscale, everything undergoes changes, leading to the formation of nanoparticles (NPs) from diverse materials due to uneven electrical dispersion (I. Khan et al., 2019)). For instance, when reduced to nanoparticles, FeAl, which is not magnetic in bulk, starts to become magnetic (NPs). Pd and Au are additional examples (Hori et al., 1999). In bulk materials, the magnetic characteristics are primarily determined by factors like magnetic anisotropy, crystallographic structure, vacancy defects and composition. On the nanoscale, however, two additional crucial parameters, size, and shape, play a significant role in influencing the magnetic properties (Jun et al., 2008). Superparamagnetism is a fascinating size-dependent property exhibited by nanoparticles (NPs). Reduction in magnetic anisotropy energy per NP is also observed as the NP size decreases. The energy of magnetic anisotropy is what maintains the direction of the magnetic moment (Skumryev et al., 2003). Each type of nanoparticle has a specific size where the anisotropic energy and thermal energy are balanced, permitting the magnetic moment to randomly reorient. This phenomenon categorizes nanoparticles as superparamagnetic, as described by (Kolhatkar et al., 2013). Superparamagnetic nanoparticles exhibit considerable magnetization when exposed to a magnetic field, but this magnetization diminishes entirely upon the removal of the field. The shape of nanoparticles is another crucial factor influencing their magnetic properties. The level of research being conducted on substantial impact of shape on the magnetic properties of NPs with the same volume, however, is far less than that on the size parameter (Hu et al., 2019).

2.4.4 Optical and Electronic Properties

Superparamagnetic nanoparticles (NPs) exhibit strong magnetization whilst a magnetic field is present, but when the field is removed, they lose all magnetizations. The second key aspect affecting the magnetic properties of NPs is their shape or form. However, there is much less study being done on the effect of shape on the magnetic characteristics of NPs with the same volume than there is on the size parameter (Kumbhakar et al., 2014). The size-dependent UV-visible extinction band that distinguishes metal nanoparticles (NPs) from bulk metals is conspicuously lacking from their spectrum. Normally, the size, shape, and dielectric environment of NPs influence their optical properties. According to studies on Ag NPs, the diameters of the particles significantly affect their optical properties. For instance, Ag NPs with a radius of 60 nm produced

a completely different response than Ag NPs with a radius of 30 nm, which displayed a major extinction peak at 369 nm (Khlebtsov & Dykman, 2011).

2.4.5 Catalytic Properties

Chemical catalysis is an increasingly expanding field, encompassing nano-catalysis that utilizes nanoparticles (NPs) as catalysts. When contrasted with their bulk counterparts, nanoparticle (NP) catalysts display notably heightened or distinct catalytic traits, such as heightened reactivity and selectivity. Multiple factors contribute to the catalytic prowess of NPs, encompassing dimensions, interparticle spacing, oxidation state, morphology, content, and the supporting structure (Cuenya, 2010). Extensive research has proven the importance of nanoparticle (NP) size on catalytic activity. A significant inverse connection has been discovered, demonstrating that smaller NPs have higher catalytic activity. This connection was demonstrated by the electro-catalytic oxidation of CO utilizing size-specific Au NPs (1.5, 4, and 6 nm) supported on indium tin oxide. Research outcomes indicated that the smallest nanoparticles yielded the most elevated normalized current densities, as highlighted in the work by (Cuenya et al., 2003). This relationship has been validated by several other studies as well. Regarding composition, numerous investigations have underscored that the incorporation of alloys into nanoparticles (NPs) can amplify their catalytic efficacy by altering the catalyst's electrical properties, mitigating detrimental effects, and introducing distinct selectivity (Shao et al., 2011).

2.5 Approaches and Methods for Nanoparticle Synthesis

Nanomaterials are produced using a variety of techniques, which may be generally divided into two categories: bottom-up and top-down procedures. Nanostructures are constructed using a bottom-up strategy from smaller building blocks like atoms, molecules, or clusters. Because of numerous electrostatic forces, van der Waals forces and other short-range forces, these atoms or molecules compress into nanometer-sized particles (Abid et al., 2022). The bottom-up method is most often used in the chemical synthesis of nanoparticles. The key advantages of the bottom-up approach include the production of a wide range of nanoparticles with scale sizes ranging from extremely small to large as well as a more uniform particle size distribution (Arole & Munde, 2014). Some bottom-up strategies for the production of nanomaterials include:

- Chemical vapor deposition
- Wet chemical synthesis

- Molecular beam epitaxy
- Sol-Gel method
- Spray conversion processing
- Physical vapor deposition

The production of nanostructured material from large bulk materials involves the employment of size reduction strategies, such as top-down or physical operations. One advantage of the top-down technique is the capacity to synthesis enormous amounts of materials. However, this approach makes it challenging to regulate form and size (Tripathy et al., 2023). This method is often helpful in the creation of nanostructured bulk materials rather than creating nanoparticles.

The following are some examples of top-down methods for synthesizing nanomaterials:

- Nanolithography
- Nanofabrication
- High pressure torsion
- Mechanical alloying

2.5.1 **Biological method/biosynthesis**

Biosynthesis represents an ecologically sound and environmentally responsible process for creating non-toxic nanoparticles that come from biological sources. Green methods for nanoparticle synthesis result in products with unique and enhanced features that are suitable for a variety of biological applications. This process uses biological templates, various plant parts, and microorganisms (including algae, fungus and bacteria) (Kolahalam et al., 2019).

2.5.1.1 **Biosynthesis method using microorganisms.**

Algae, bacteria and fungi offer viable pathways for producing an extensive array of nanomaterials from aqueous solutions containing metal salts. For instance, Under anaerobic circumstances, magnetotactic bacteria found on the ocean floor may biosynthesize magnetic particles. Photosynthetic bacteria such as *Rhodopseudomonas capsulata* contribute to the production of extracellular gold nanoparticles sized between 10 to 20 nm. *Fusarium oxysporum*, a fungus, is harnessed for extracellular synthesis of silver nanoparticles, while *Sargassum wightii* algae serve in generating extracellular gold nanoparticles (Gahlawat & Choudhury, 2019). However, it is

noteworthy to consider that the use of this strategy must be done with extreme caution since some bacteria, fungus, and algae might have harmful features.

2.5.1.2 **Biosynthesis method utilizing plants and plants parts**

Exploration into nanoparticle synthesis via plants and their extracts has gained considerable attention. The presence of phytochemicals within plants assumes a fundamental role in the reduction of metal nanoparticles. Among the natural reducing agents utilized for nanoparticle synthesis, flavones, organic acids, and quinones feature prominently. The abundant variety and convenient availability of plant resources have been thoroughly investigated for their potential in nanomaterial production (Mondal et al., 2011). Recent research has showcased the successful biogenic synthesis of nanoscale particles, wires, floral structures, and tubular forms. These biologically originated nanomaterials carry significant promise for applications across diverse domains, encompassing medical treatments, diagnostic techniques, advancements in surgical nanodevices, and the manufacturing of commercial products (Bar et al., 2009). Utilizing biomass from plants like geranium (*Pelargonium graveolens*) and alfalfa (*Medicago sativa*) various forms of gold nanoparticles have been generated (Parsons et al., 2007). *Azadirachta indica* (neem) leaves contribute to the production of bimetallic Au, Ag, and core-shell nanoparticles of Au with an Ag shell. The extract from aloe vera leaves is employed to create gold nano triangles. Additional plants, including *Brassica juncea*, *Helianthus annuus*, and sunflower, are leveraged for generating nanoparticles of silver, nickel, cobalt, zinc, and copper (Rai & Yadav, 2013).

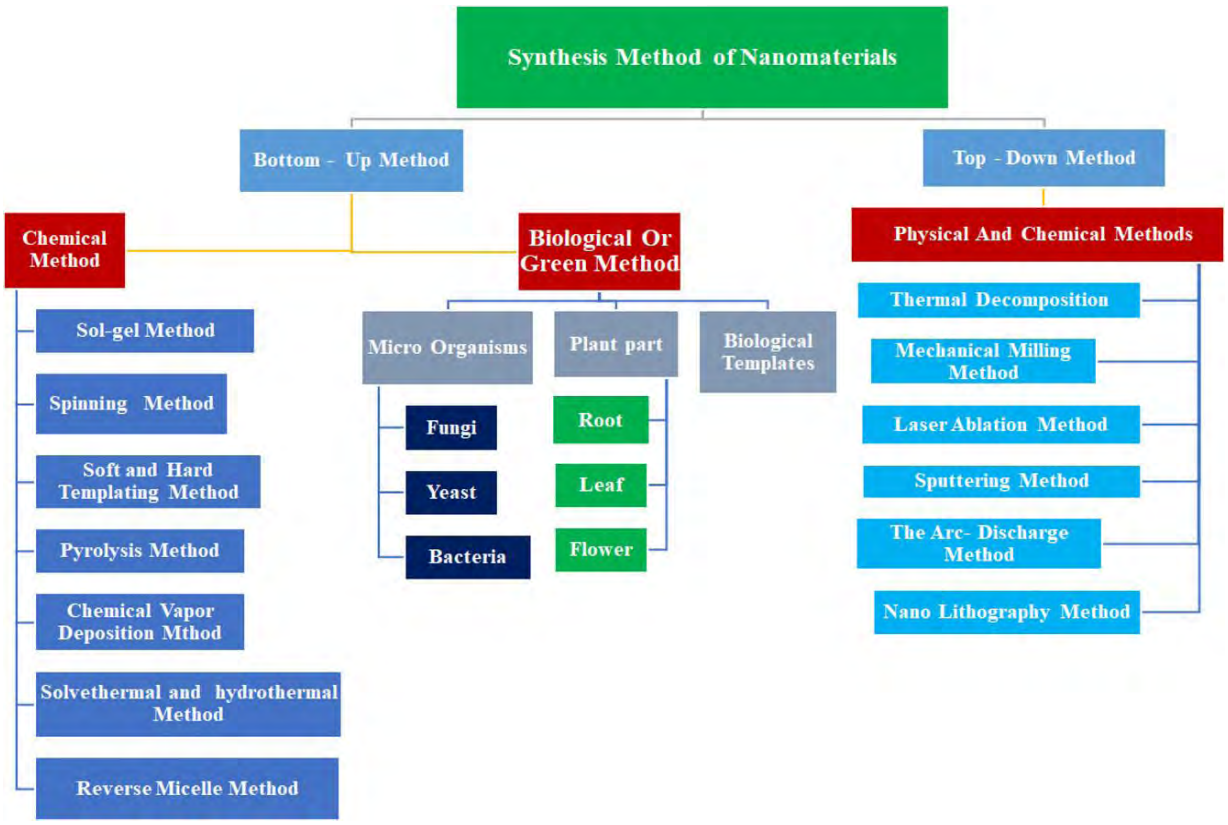


Figure 2.1: Various methods for nanoparticle synthesis (Mekuye & Abera, 2023)

2.6 Biological Synthesis of Copper Oxide Nanoparticles

Copper (Cu) holds pivotal importance as a trace element essential for the well-being of animals, humans, and plants (Raha et al., 2020). While humans require copper in minute quantities, a typical adult with an average weight of approximately 70 kg retains around 100 mg of copper within their body (Shabbir et al., 2020). The recommended daily intake of copper ranges from 2 to 4 mg, with a permissible limit of up to 10 mg from dietary sources, encompassing food and beverages. In addition to acting as a vital cofactor for a myriad of enzymes that contribute in the manufacturing of neuropeptides, copper also plays a significant role in the regulation of cell signaling pathways, acts as an antioxidant defense mechanism, and supports immune cells that are essential for human pathogen defense (Waris et al., 2021). Copper is a trace element that is needed for the development of plants and plays a crucial role in many biochemical and physiochemical activities (Ghaderian & Ravandi, 2012). It acts as a cofactor for several enzymes and is necessary for the correct functioning of a variety of important proteins and enzymes, including plastocyanin, amino oxidase, and cytochrome c oxidase (Sifri et al., 2016).

Copper oxide (CuO) nanoparticles are gaining popularity among nanoparticles due to their numerous applications (Krishnan & Mahalingam, 2017). With a bandgap of just 1.7 eV and a monoclinic structure, copper oxide is a p-type semiconductor (Rafea & Roushdy, 2008). Copper oxide nanoparticles have a wide range of characteristics and uses. Copper oxide finds applications in various fields, including biomedical applications such as antimicrobial, anti-fouling, antifungal, antibiotics, antioxidants, drug delivery, and anticancer agents. It is also used in the textile industry, gas sensors and catalytic processes. Additionally, copper oxide plays a role in magneto-resistant materials, high-temperature superconductors, environmental remediation, and other diverse applications (Verma & Kumar, 2019). Numerous physical-chemical processes have been used to produce copper oxide nanoparticles. However, there are several drawbacks to these methods, including the release of numerous highly hazardous substances into the environment, excessive energy consumption, and expensive costs (Buazar et al., 2019). As a result, a process that is environmentally benign, more sustainable, and economically viable is needed to create nanoparticles that are homogeneous in particle size, shape, and texture and have high purity, crystallinity, phase selectivity, (Rehana et al., 2017). The creation of an eco-friendly and low-cost technique for making nanoparticles is the product of green chemistry and other biological processes (A. Singh et al., 2017). Bio-mediated nanoparticle production is an environmentally benign, dynamic, safe, and cost-effective technique (Rehana et al., 2017). Numerous essential components found in green resources are needed for the reduction, chelation, and stabilization, including various metabolites (phenolic compounds, lipids, proteins, enzymes, sugars, and polysaccharides), as well as functional groups like polyols, carboxylic acids and amino groups (Ovais et al., 2018).

2.6.1 Copper Oxide Nanoparticle Synthesis Using Plants

The use of plant extracts has been widely employed for producing CuO-NPs (Prakash et al., 2018). Although the production of CuO-NPs from algae, bacteria and fungus has many benefits, there are also some disadvantages (F. Ijaz et al., 2017). The main challenges are the separation of microorganisms, bacterial toxicity and the incubation process. Plant extracts thus offer a great source of nanoparticles made of metals and metal oxides (Bordbar et al., 2017). According to (Rajesh et al., 2018), plant-based manufacturing is a secure and simple process that uses less energy and produces particles that are more stable. The reaction in this procedure, which starts when the metal salt and plant extracts are joined, concludes within a period of one to three hours

at room temperature. Flavonoids, phenols, proteins, terpenoids, and tannins are just a few of the bioactive metabolites found in plant extracts. These compounds act as stabilizers and reducers, enabling the formation of nanoparticles from metallic ions (Asemani & Anarjan, 2019). The plant extract generates electrons that compel copper salts to reduce. The interaction of phytoconstituents with copper ions result in reduction, which in turn produces copper oxide nanoparticles (Mali et al., 2019).

Copper nanoparticles have an array of potential applications and may be produced using environmentally friendly techniques and a variety of medicinal plants. The production of copper nanoparticles using plant extracts abundant in bioactive chemicals has been investigated in a number of publications. For instance, copper nanoparticles with potential medicinal uses have been made using the peel extract of *Punica granatum*, a plant recognized for its immunomodulatory effects (Ghidan et al., 2016). Similar to this, copper oxide nanoparticles with remarkable antibacterial and antioxidant activities have been made using the leaf extract of *Calotropis procera* (Sukumar et al., 2020). Additionally, when utilized in the environmentally friendly synthesis of copper nanoparticles*, Cynodon dactylon* and *Cyperus rotundus* grass extracts have demonstrated potential as antibacterial agents (Vaidehi et al., 2018) . It has also been reported by (Prakash et al., 2018) that *Cordia sebestena* flower extract may be used to synthesize CuO-NPs. As reported by (Bordbar et al., 2017), *Rheum palmatum* L root extract was effectively used to create copper oxide nanoparticles. These nanoparticles had spherical morphologies and were between 30 and 50 nanometers in size. These examples demonstrate how medicinal plants may be used in a variety of ways to produce copper nanoparticles with beneficial effects ranging from antibacterial and antioxidant to immunomodulatory, paving the door for novel uses in both industry and medicine.

2.6.2 ***Echinacea purpurea*- The Plant of Interest**

Echinacea purpurea (L.) Moench, a vigorous and enduring perennial herbaceous plant, reaches a height of 100-150 cm. Its cylindrical roots exhibit a brownish-grey outer layer and a white inner core. The branching aerial stem bears coarse hairs and reddish-brown marks, giving it a bush-like appearance. During the initial year of cultivation, it forms a basal cluster of leaves but only comes into bloom during its second year (Mistr\'\iková & Vaverková, 2007). Inflorescences hold clustered flowers, and the terminal anthodes exhibit a range of hues from pink to pale crimson to deep red. As the inflorescence grows, rigid bracts near its base lignify and develop thorny

protrusions. Sterile ray flowers in shades of pink are situated along the margin of the inflorescence, with ligules measuring 0.5 cm in width and 5-7 cm in length. Tubular disc flowers with an orange-brown tint, bisexual in nature, are nestled within the inflorescence. The achene fruits, whitish grey in color, possess four edges and are crowned with teeth. *Echinacea purpurea* blooms from June to July (Belaeva & Butenkova, 2018).

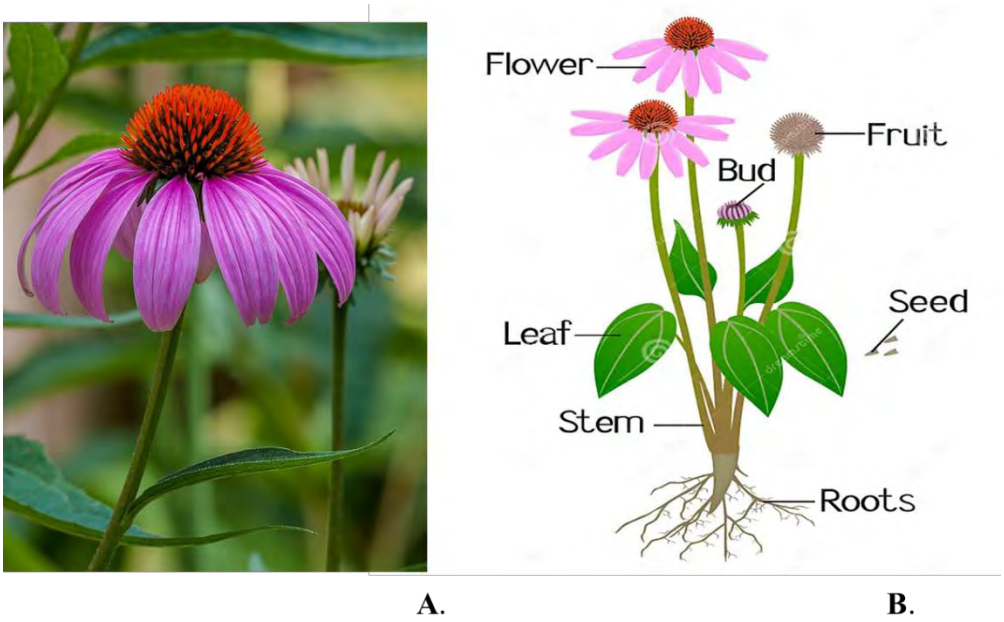


Figure 2.2: A . *Echinacea purpurea* (purple cone flower) (https://www.wildflower.org/gallery/result.php?id_image=45219) **B**. Labelled diagram of *Echinacea purpurea* (https://www.dreamstime.com/illustration/echinacea-purpurea.html?pg=3)

Echinacea purpurea naturally grows in damp prairies, meadows, and open woodlands across the central to southeastern regions of the United States, spanning from Ohio to Michigan to Iowa, and extending southwards to Louisiana and Georgia. This plant is indigenous to various areas of eastern North America and is found in the wild to some extent across the Eastern, Southeastern, and Western parts of the United States, along with the Canadian province of Ontario (A. Singh et al., 2022).

Echinacea purpurea has various names and synonyms based on their botanical, horticultural and medicinal records. Some of their common names and synonyms that are used earlier in history are Purple coneflower, Eastern purple cone flower, Red sunflower, Hedgehog coneflower Rudbeckia, Brauneria purpurea (L.), Rudbeckia purpurea, *Echinacea purpurea* var. arkansana Steyerm (Kindscher, 2016; Kindscher & Wittenberg, 2016)

The scientific classification of the plant is as follows:

Table 2.1: Classification of *Echinacea purpurea* (Kindscher & Wittenberg, 2016)

KINGDOM:	**PLANTAE**
PHYLUM:	Anthophyta
CLASS:	Dicotyledoneae
ORDER:	Asterales
FAMILY:	Asteraceae
GENUS:	Echinacea
SPECIES	*Echinacea purpurea*

Echinacea purpurea (L.) Moench comprises crucial constituents like alkylamides, polysaccharides, glycoproteins, flavonoids, and phenolic compounds, including derivatives of caffeic acid such as chicoric acid, caftaric acid, chlorogenic acid, and echinacoside (Attarzadeh et al., 2020). The proportions of these components vary across different plant segments. In addition to these elements, a consistent presence of phylloxanthobilins, - dimethyl sulphide, acetaldehyde, camphene, limonene, hexanal and phellandrene-pinene was seen all across the plant, regardless of species. The existence of fatty acids along with aldehydes and terpenoids is contingent on the specific parts of the plant used (de Oliveira et al., 2021). Echinacoside, identified in a concentration of 1.45 percent in the flower, has been found to offer several pharmacologically significant health benefits, encompassing neuroprotective and cardiovascular properties (Brown et al., 2010; Tabar et al., 2019).

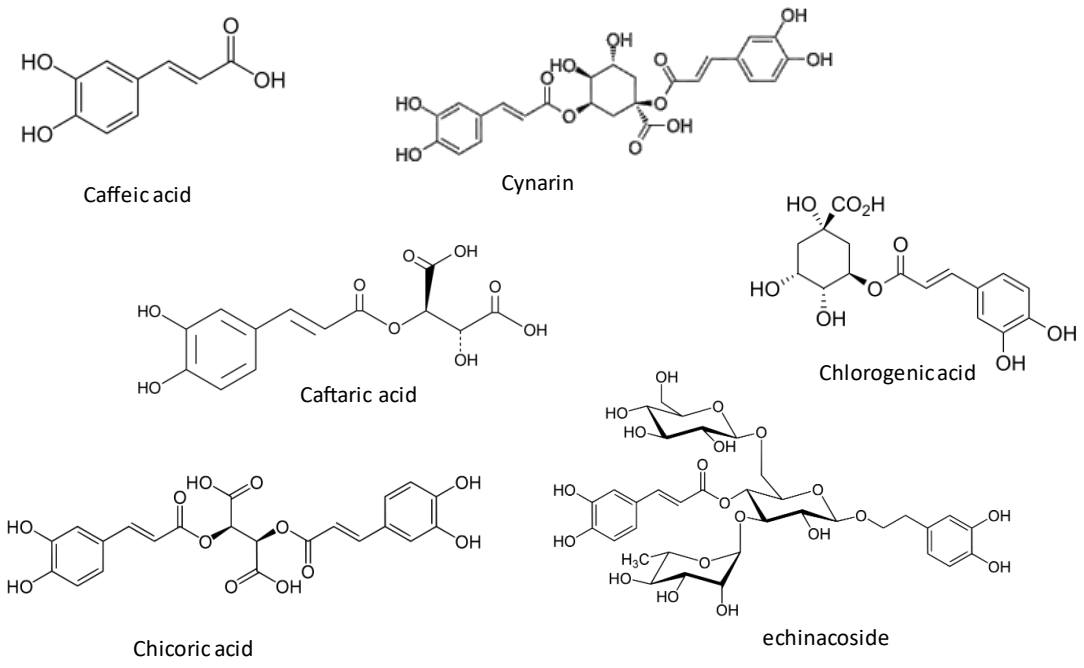


Figure 2.3: Chemical structures of secondary metabolites found in *Echinacea purpurea* (Sharifi-Rad et al., 2018).

Echinacea species also contain flavonoids, polyacetylenes, and alkaloids in addition to these substances (Jäger et al., 2022). *Echinacea purpurea* (L.) Moench leaf extracts have revealed the presence of significant constituents called phylloxanthobilins. These natural tetrapyrrole compounds originate from the degradation of chlorophyll. While discovered approximately a decade ago in the leaves of deciduous trees, phylloxanthobilins are now recognized as a compound class with notable potential for high bioactivity, which remains largely unexplored. Additionally, To treat skin and wound irritation, *Echinacea purpurea* (EP) is being developed as a topical drug. Moreover, Echinacea-based medications have been approved in Europe for the treatment of upper respiratory tract infections and wound healing (Billah et al., 2019). Recent pharmacological investigations have unveiled various bioactivities attributed to EP, including its immunomodulatory, anti-inflammatory, antioxidant, antiviral, and antifungal properties (Senchina et al., 2010). EP has been suggested for potential therapeutic use in addressing conditions such as chronic arthritis, cancer, antibacterial effects, chronic fatigue syndrome, HIV infection, assorted skin disorders, wound management, and persistent pelvic infections(Soon & Crawford, 2001).

Echinacea purpurea, a well-known medicinal herb, demonstrates exceptional promise in the biosynthesis of different nanoparticles, paving the way for new nanotechnology applications

(Annu & Ahmed, 2018). Researchers are growing interested in this herbaceous plant's unusual qualities, particularly its potential to generate nanoparticles in an environmentally acceptable and sustainable manner. *Echinacea purpurea* contains an array of active compounds, encompassing polyphenols, flavonoids, and alkaloids, which function as inherent stabilizers and reducers in the process of nanoparticle synthesis (Stanisavljević et al., 2009). Among the noteworthy nanoparticles that can be biosynthesized utilizing *Echinacea purpurea* are silver nanoparticles (AgNPs). These NPs were synthesized by treating $AgNO_3$ solution with *Echinacea purpurea* extract, which resulted in the formation of very stable AgNPs by reduction of silver ions with numerous uses in medicine, catalysis, and antimicrobial coatings (Fierascu et al., 2022). This extraordinary plant can also be used to create gold nanoparticles (AuNPs). These AuNPs have proven remarkable biocompatibility and unique optical features, making them important assets in biomedical research targeted drug delivery and imaging modalities. Biosynthesis of zinc oxide nanoparticles (ZnONPs) with regulated form and size has been demonstrated using *Echinacea purpurea* (Karimi et al., 2018). These ZnONPs have excellent antibacterial and UV-blocking capabilities, making them useful in sunscreens and antimicrobial coatings (Attar & Yapaoz, 2018). Another notable example is the creation of iron oxide nanoparticles (Fe-NPs) from *Echinacea purpurea* extract, which have shown enormous potential in MRI contrast agents, hyperthermia-based cancer therapy, and environmental remediation (Al-Hakkani et al., 2021).

In addition to metallic nanoparticles, this herbal plant has been shown to produce semiconductor nanoparticles such as cadmium sulphide (CdS) and quantum dots, which have potential applications in optoelectronics, photocatalysis, and solar cells (Regmi et al., 2023). The biogenic synthesis of nanoparticles using *Echinacea purpurea* has significant advantages over traditional chemical approaches since it is a green and sustainable technology that is devoid of toxic chemicals and hazardous by-products. Using *Echinacea purpurea*'s unique bioactive components for nanoparticle synthesis not only demonstrates the plant's astonishing adaptability, but also lays the door for the production of innovative and eco-friendly nanomaterials with wide applications ranging from biomedicine to environmental research (Soufi & Iravani, 2020).

2.6.3 Applications of Copper Oxide Nanoparticles in Biomedicine

2.6.3.1 Antibacterial Activity

Published studies have shown that CuO-NPs are extremely dangerous for the majority of human disorders (Applerot et al., 2012). Bio-fabricated copper oxide nanoparticles have garnered significant attention from researchers as a promising avenue for antibacterial interventions. This enthusiasm stems from their distinct morphologies, sizes, and biocompatibility, which collectively enable them to effectively target a diverse spectrum of pathogenic human bacteria (Awwad et al., 2015). Scientists have reported the strong antibacterial properties of green-produced copper oxide nanoparticles against both Gram-positive and Gram-negative bacterial pathogens. The nanoparticles were manufactured using *Tabernaemontana divaricate* leaf extract, and their antibacterial effectiveness against pathogens responsible for urinary tract infections (UTIs) was investigated. Escherichia coli was the target of the most potent inhibitory zone, which, at a dosage of 25 µg/ml, had an average diameter of 17 mm (Sivaraj et al., 2014). Copper oxide nanoparticles initiate the generation of reactive oxygen species (ROS) that interact with bacterial cell membranes, facilitating their penetration into the cells. Consequently, this process disrupts the cell membrane, leading to the inhibition of bacterial cell growth and potentially culminating in cell death (Das et al., 2013). Additionally, copper oxide nanoparticles disrupt proteins and DNA, prevent the growth of biofilms, damage proton efflux pumps, and cause bacterial cellular components to oxidize (Akintelu et al., 2020). Although a possible process is shown in Fig. 2.4, the specific method by which copper oxide nanoparticles cause toxicity to bacterial strains is unclear.

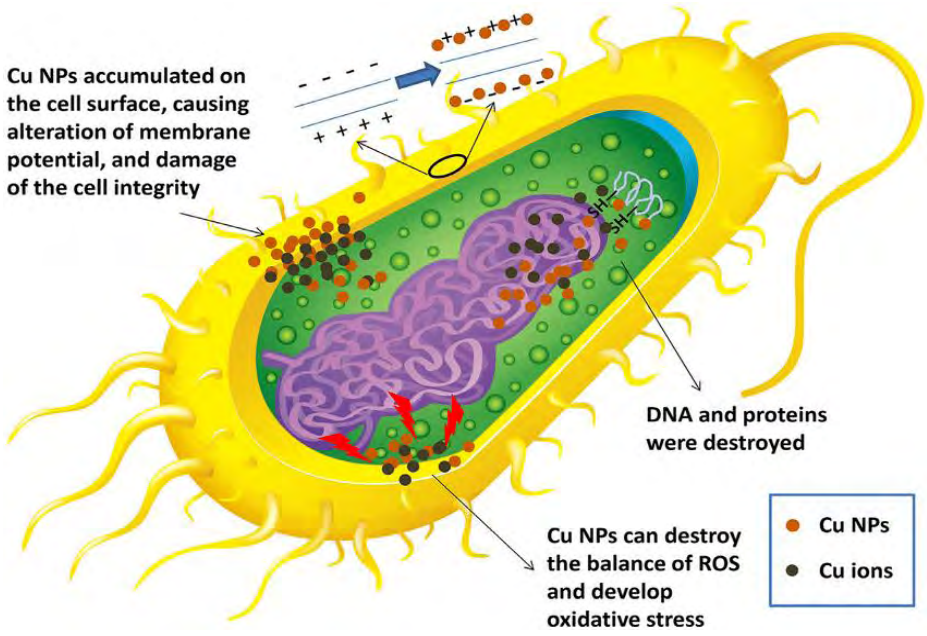


Figure 2.4: Analyzing a putative mechanism for CuO-NPs toxicity against Bacteria (Makvandi et al., 2020)

2.6.3.2 **Antifungal Activity**

The antifungal potential of CuO-NPs has been inspected for medicinal applications to treat fungus infections (Rajesh et al., 2018). While copper oxide nanoparticles' antibacterial activities have attracted a lot of interest, their antifungal effects have gotten comparably little attention. Researchers have delved into the utilization of the fungal strain *Penicillium chrysogenum* for the microbial synthesis of CuO-NPs. These eco-friendly NPs were tested for efficacy against harmful fungi strains, including *Fusarium oxysporum*, *Aspergillus niger*, *Penicillium citrinum* and *Alternaria solani*. According to the study's findings, all these strains were all successfully inhibited by green-synthesised copper oxide nanoparticles, with zones of inhibition measuring 37.0 ± 0.76376, 28.0 ± 0.86603, 26.5 ± 0.76376, and 20.7 ± 0.43589, respectively (El-Batal et al., 2020).

2.6.3.3 **Anticancer activity**

Nanoparticles exhibit dimensions on a nanoscale, distinguishing them from larger biological entities such as enzymes and receptors. They exhibit distinct morphologies, stability, and unique interactions with biomolecules, which hold promise for their potential applications in cancer treatment and diagnosis (Kouhkan et al., 2020). The anticancer properties of several types of nanoparticles have been studied and published (Kelkawi et al., 2017). Bioinspired copper oxide

nanoparticles, like other nanoparticles, have anticancer properties against several cancer cell types. Copper oxide nanoparticles act against cancer cell lines through various mechanisms, including inducing chromosomal aberrations and DNA breakage. They engage with intracellular macromolecules, prompting apoptosis and eventual cell demise. Moreover, copper oxide nanoparticles possess the capability to disrupt membrane functionality, inducing cellular leakage (Akintelu et al., 2020). Using the SRB assay, the anticancer potential of CuO-NPs synthesized from dried black beans were assessed in human cervical carcinoma cells. The Hela cell line was cultured within 96-well plates, subjected to varying copper oxide nanoparticle concentrations for durations of 12, 24, and 48 hours. The investigation unveiled the nanoparticles' cytotoxic impact on Hela cells, with concentrations of 1 mg/ml and 0.5 mg/ml of CuO-NPs impeding cell proliferation. Notably, copper oxide nanoparticles hindered cervical cancer colonies by modulating the production of intracellular ROS in a dose-responsive manner. Furthermore, the scientists carefully examined changes in morphology of nucleus and mitochondria following exposure to CuO-NPs (Nagajyothi et al., 2017).

2.6.3.4 **Other Applications of Copper Oxide Nanoparticles**

Biogenic copper oxide nanoparticles not only showcase attributes like antibacterial, antifungal, anticancer, and antiviral effects but also hold potential for broader applications in diverse diseases (Yugandhar et al., 2017). Furthermore, bioinspired copper oxide nanoparticles have demonstrated larvicidal capabilities. In the study conducted by (Muthamil Selvan et al., 2018), Tridax Procumbens leaf extracts were used to create copper oxide NPs in an eco-friendly manner. The investigation extended to assessing the larvicidal potency of these nanoparticles against the *Aedes aegypti* mosquito, which serves as the vector for diseases such as dengue, chikungunya, and zika. According to research findings of (Muthamil Selvan et al., 2018), CuO-NPs are incredibly effective at repelling *Aedes aegypti* mosquitoes. A plant extract from *Abies spectabilis* was used by (H. Liu et al., 2020) to demonstrate the biofabrication of CuO-NPs. The positive potential of these green nanoparticles for anti-inflammatory and antinociceptive effects was highlighted in their study. To fully comprehend the specific mechanism by which copper oxide nanoparticles treat inflammatory illnesses, however, more investigation is necessary (H. Liu et al., 2020).

2.6.4 Factors affecting synthesis of Plant-Extract based Copper Oxide Nanoparticles

2.6.4.1 Solution pH

The pH of a solution is an important aspect to consider while biosynthesizing CuO-NPs from plant sources (Gericke & Pinches, 2006). On the synthesis time, size, and shape of generated nanoparticles, the impacts of solution medium pH have been highlighted (Vijayaraghavan & Ashokkumar, 2017). During the synthesis of nanoparticles, the pH level significantly affects the growth of nucleation sites. A higher pH can lead to an increased number of nucleation centers, promoting the reduction of metallic ions (such as copper ions) into metal nanoparticles (CuO-NPs). The interaction between metal ions and functional groups in plant extract is significantly influenced by pH, which has an impact on the reduction time period of the metal salt during the process (Bali & Harris, 2010). Scientific studies have indicated that smaller-sized nanoparticles are more likely to form in a basic medium compared to an acidic solution. For instance, CuO-NPs synthesized at pH levels of 3, 5, and 7 exhibited surface plasmon resonance (SPR) band peaks at 600, 590, and 584 nm, respectively (S. Wu, 2007).

2.6.4.2 Reaction time

The incubation and reaction times have a major influence on the quality, structure, and yield of CuO-NPs (Kuchibhatla et al., 2012). The characteristics of the generated nanoparticles are influenced by variations in incubation time as well as storage conditions (Mudunkotuwa et al., 2012). Several data indicate that extensive response times are necessary for successful nanoparticle synthesis (Darroudi et al., 2011) . The formation of CuO-NPs was UV analyzed, and the results showed a decrease in absorption that began at 2 hours and peaked at 4 hours. The intensity of the absorption peaks grew with longer reaction times until stabilizing at three hours, demonstrating the stability of the produced Cu-NPs (Pham et al., 2019).

2.6.4.3 Temperature

Temperature is another crucial agent that influences the formation of CuO-NPs; it has a similar impact on the morphological characteristics as pH does. Temperature also plays a significant role in the creation of nucleation centers; lowering the temperature leads to a reduction in nucleation center formation, thereby decreasing the synthesis rate (Pham et al., 2019). Considering that the plant extract contains secondary metabolites, room temperature is considered the optimal temperature for nanoparticle formation to inhibit the degradation and alteration of the functional

groups (Jemilugba et al., 2019). However, research has shown that spherical nanoparticles are created at higher temperatures than triangular nanoparticles, which are synthesised at lower temperatures (Raju et al., 2011).

2.6.4.4 **Effect of plant extract concentration**

The concentration of plant extract plays a vital role in supplying electrons for the reduction of copper ions, thereby influencing the production of CuO-NPs. Lowering the quantity of plant extract results in a corresponding decrease in the generation of CuO-NPs, as observed by Kiruba (Kiruba Daniel et al., 2013). On the other hand, utilizing a larger volume of plant extract during the biosynthesis of CuO-NPs yields a greater quantity of phytochemicals, promoting the rapid reduction of copper salt. However, this approach also leads to the formation of smaller Cu-NPs due to the heightened availability of reducing agents (Din & Rehan, 2017).

2.6.4.5 **Concentration and nature of copper salt**

Each type of copper salt employed to synthesize CuO-NPs has a substantial impact on their nature, structure, and size. Copper salts such as copper nitrate, copper sulphate, copper chloride, and copper acetate are the most often utilized copper salts for the creation of nanoparticles. For instance, using copper chloride salt results in the formation of triangular and tetrahedron-shaped CuO-NPs, while copper acetate salt yields rod-like CuO-NPs (Shankar & Rhim, 2014). On the other hand, spherical CuO-NPs are produced when copper sulphate salt is used (M. Shah et al., 2015). Moreover, an increase in the concentration of copper salt leads to larger-sized CuO-NPs, as observed in one study (Din & Rehan, 2017).

CHAPTER 3

MATERIALS AND METHODS

3. Materials and Methods

All the experiments were performed under the supervision of Prof. Dr. Bilal Haider Abbasi at Plant Cell and Tissue Culture Laboratory, Department of Biotechnology, Quaid-i-Azam University Islamabad.

3.1 Chemicals and Equipment

In the current study, the following chemical compounds were utilized: distilled water, sodium hydroxide, methanol, hydrochloric acid, ethanol and mercuric chloride. Plant growth regulator (TDZ) was used to prepare callus cultures of *Echinacea purpurea* and Copper Oxide Pentahydrate ($CuSO_4.5H_2O$) was used to prepare salt solution. The equipment utilized for this study include filter paper, a spatula, a blade, forceps, falcon tubes, Eppendorf, an autoclave (KP-30L, ALP Tokyo Japan), an electric balance (GF-300), a pH meter (Jenway 3305), a laminar flow transfer cabinet (ESCO), a magnetic stirrer, a vortex, a centrifuge, an incubator a burner, and glassware (Measuring cylinder, Erlenmeyer flask, glass beaker, petri dish) etc.

3.2 Surface Sterilization

To remove dust particles and other contaminants from the glassware and utensils used in the study, they were meticulously washed with running water and detergent. After thorough drying, the items were securely wrapped in paper and subjected to sterilization in an autoclave at 15 psi pressure and 121°C for approximately twenty minutes.

3.3 Media Preparation

The germination of seedlings of *Echinacea purpurea* took place in Murashige and Skoog basal medium (MS, 1962). Media preparation followed the protocol specified by (Abbasi et al., 2010). In order to do this, 30g of the sucrose and 4.4g of the MS medium were weighed in a weighing scale before being dissolved in distilled water in a flask to generate a total amount of 1 liter. The medium pH was kept at 5.65± 0.02 by use of (1.0 N) HCL and (1.0 N) NaOH. The media were then solidified by adding 8g of agar, which was weighed before being added. After that, flasks were put inside the microwave for 5 minutes for agar to get agar boiled and dissolved effectively. Each 100-ml Erlenmeyer flask was then filled with 40ml of the medium and firmly sealed using cotton and aluminum foil. To guarantee proper solidification of the medium and eliminate any

potential contamination, the flasks underwent sterilization through autoclaving, with each flask subjected to 20 minutes at 15-psi pressure and at 121 degrees celcius. Subsequently, the sterilized flasks were allowed to stand overnight.

3.4 Collection of Explant, Inoculation and Seed Germination

Echinacea purpurea seeds were procured in perfect health and uniformity from University of Tours, France by Dr. Bilal Haider Abbasi in March 2022. In a laminar flow (LFH) cabinet with a HEPA filter, inoculation was carried out in a sterile setting. Following this, an autoclaved flask holding 40ml of the medium was moved to LFH along with autoclave tools like a Petri dish, forceps, blade, distilled water, empty beaker, and ethanol. To lessen the risk of contamination, all of these devices were surface sterilized with 70% ethanol. For effective sterilization, the LFH door was shut and a UV lamp (GKL-511, 50 Hz, 19w) was turned on. After opening the LFH, turned off the UV light, and the fans were turned on. Hands were sanitized with 70% ethanol prior to vaccination. Placed the flame of the spirit lamp next to the open Petri dishes with filter paper. Within the laminar flow hood, seeds were surface sterilized after being rinsed with water. They were first immersed in 0.1% $HgCl_2$ (w/v) for one minute. It was then submerged in 70% EtOH for around 40 seconds. Finally, three rinses with sterile distilled water were performed to remove any remaining dust, and the surface was then dried on sterile Whatman filter paper. There were two to three seeds inoculated per flask. Each experiment was carried out twice in duplicate. Following that, these flasks were placed in the growth chamber for 50 days in self-regulating environmental condition, such as setting the temperature to 25°C and supplying a 16/8-hour photoperiod with a fluorescence light bulb that has a 40 µmol m^{-2} s^{-1} light intensity (Philips Tornado Spiral).

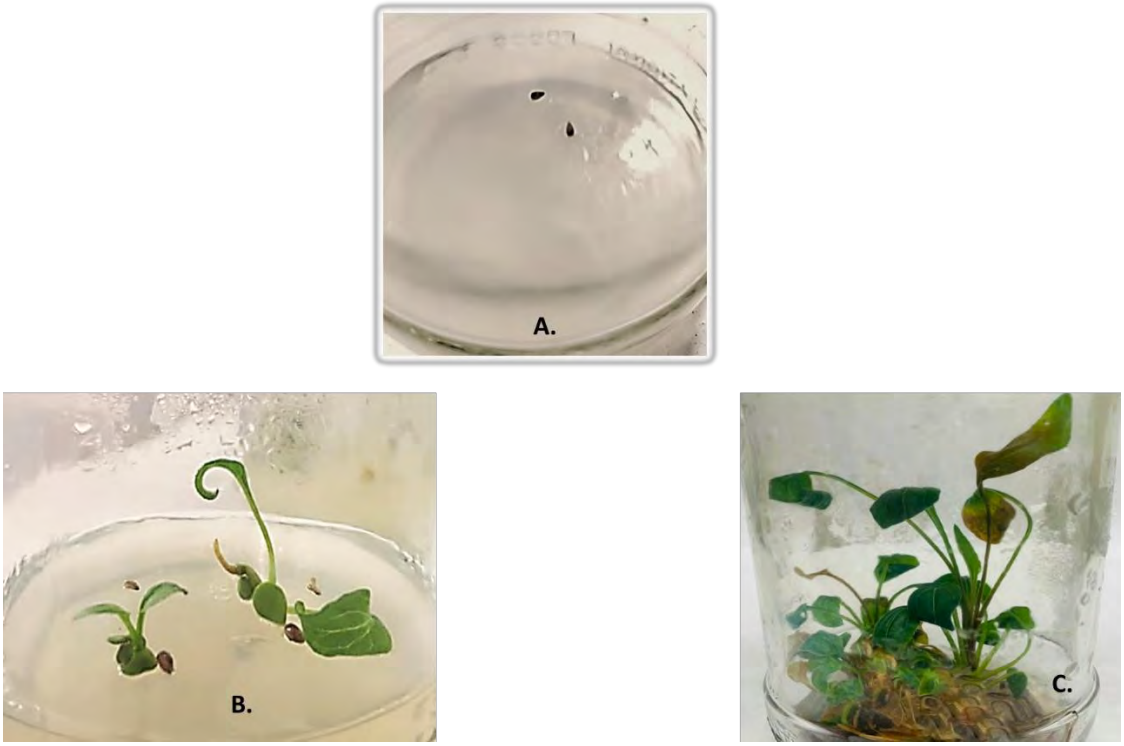


Figure 3.1: A. Seeds inoculated on MS basal media. **B**. *Invitro* seedling. **C.** *Invitro* grown plant of *Echinacea purpurea.*

3.5 Callus Establishment

Echinacea purpurea callus culture was established in the first experiment. For callus induction MS media (4.4 g/L) supplemented with sucrose (30g/L) and agar (8g/L) and 3mg/L thidiazuron (TDZ) was prepared (unpublished data). 60-day old plantlets grown (in-vitro) from seeds were chopped into small pieces, and 4-5 pieces were inoculated in each flask under LFH. Then, flasks containing infected explants were exposed to photoperiod (i.e., sixteen hours of light and eight hours of darkness) at an intensity of 40 mol m^{-2} s^{-1}. After 14–15 days, the callus began to grow. The callus had grown to its maximum size after 50 days.

Figure 3.2: Invitro grown Callus of *Echinacea purpurea* on MS media supplemented with 3mg/L TDZ by using stem explant.

3.6 Plant Extract Preparation

Calli were transferred from medium onto Whatman filter paper for the removal of excess water contents after 50 days of exposure to light. For the purpose of determining the dry weight, the fresh weight of the calli was first determined. A mortar and pestle were used to crush Callus.

In order to create copper oxide nanoparticles and perform spectrophotometric analysis on them, 100mL of distilled water was mixed with 5 grams of dried callus. The mixture was subjected to boiling for one hour at 60℃. Extract was then placed in incubator for one day at 40℃. The sample was then subjected to filtration thrice through Whatman filter paper. The supernatant was then kept in refrigerator at 4℃ for further use.

Dried calli were treated to the extraction process recommended by Zahir et al. in order to conduct antibacterial and antioxidant examination on the nanoparticles that were produced. 500µl of methanol and 0.1g of callus powder were homogenized using sonication for 30 minutes and vertexing for 15 minutes. The extraction process was done twice prior to centrifugation (10 min, 15000 rpm). The supernatant was kept at 4 °C after centrifugation, and the pallet was discarded.

Figure 3.3: Callus extract of *Echinacea purpurea*

3.7 Preparation of Salt Solution

In 100 mL of distilled water, 2.4 grams of copper sulphate pentahydrate ($CuSO_4.5H_2O$) were dissolved to create salt solution. Until the salt was entirely dissolved, the liquid was continually stirred at room temperature.

Figure 3.4: copper sulphate pentahydrate ($CuSO_4.5H_2O$) solution in water

3.8 Copper Oxide Nanoparticle Synthesis

100 mL of salt solution received dropwise additions of 40 mL of plant extract. At a temperature of 60°C, the solution was constantly agitated for 4 to 6 hours at a speed of 400 rpm. The color of the mixture changed from dark green to dark brown indicating the reduction of copper sulphate into copper oxide nanoparticles. The solution was kept overnight an in incubator at 40°C. The copper oxide nanoparticles obtained were centrifuged for 20 minutes at 3500 rpm. The pallets obtained were washed thrice with distilled water and twice with ethanol in a centrifuge machine. The final pallets obtained were poured into petri dishes and dried in incubator at 60°C for 3 hours. The

powder obtained was grinded with pestle and mortar to get fine particles of nanoparticles. The obtained copper oxide nanoparticles were then subjected to further analysis like characterization and different biological assays.

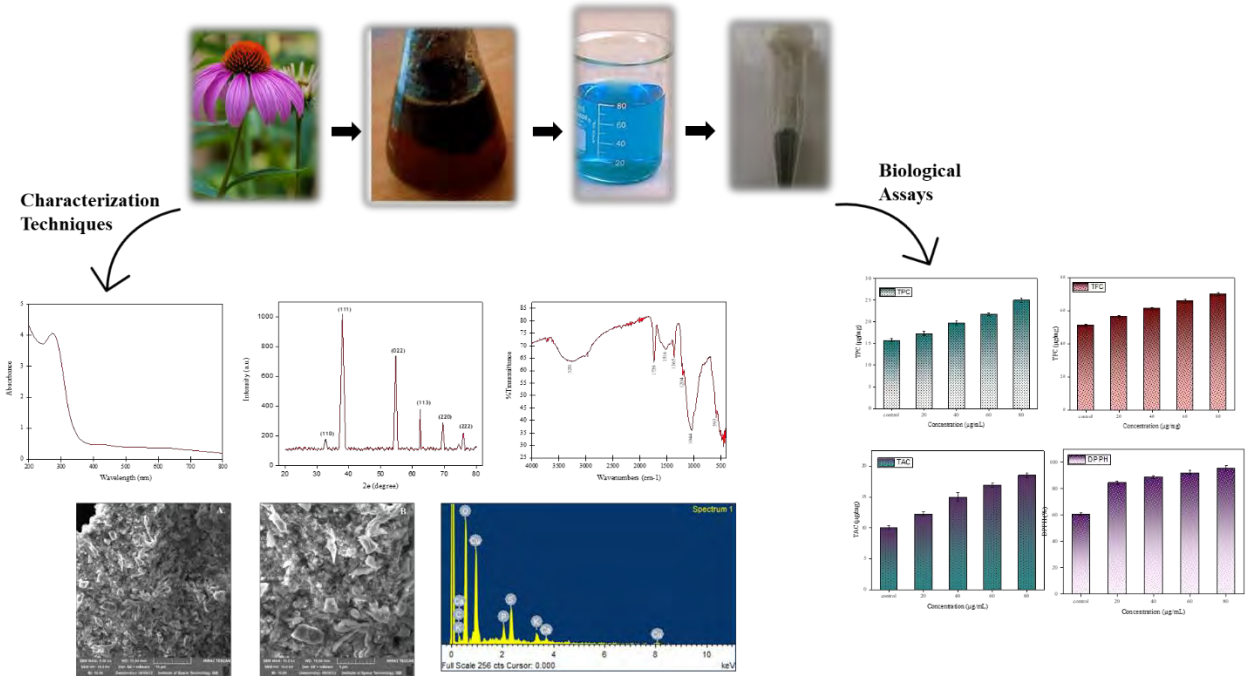


Figure 3.5: Graphical representation of synthesis, characterization and biological assays for biosynthesized CuO-NPs.

3.9 Characterization of Copper Oxide Nanoparticles

3.9.1 UV/VIS Spectrophotometry

Using a UV-Vis spectrophotometer made in Australia (UV-Vis Halo DB20), operating within the wavelength spectrum of 200 to 800 nm, the bio-reduction process and the optical properties of the decomposition of Cu ions into CuO nanoparticles were evaluated. One milliliter of distilled water was mixed with one milligram of the synthesized nanoparticles, which was then sonicated for 20 minutes before being utilized for analysis. The resultant combination was then subjected to a UV-Vis spectrophotometric examination. By adding a solution of NaOH (1 N), the mixture's pH was changed to 7.0.

3.9.2 X-ray Diffraction (XRD)

XRD analysis was used to find the crystallographic arrangement of the green-synthesised nanoparticle particles. The XRD equipment used was a (Schimadzu-Model Kyoto, Japan) XRD. The investigation used Cu/Kα radiation with a wavelength of 1.5406 and covered a 2θ range from

10° to 90°. The device maintained a constant setting at ambient temperature while operating at 40 kV and 30 mA. For calculating the particle size, the Debye-Scherer equation **($D = K\lambda/\beta\cos\theta$)** was utilized, with the following variables: θ corresponds to Bragg's angle, λ represents the X-ray wavelength (1.5406 Å), β signifies the angular full width at half maximum in radians, D indicates the crystal size perpendicular to the reflecting planes, and K stands for the constant (0.9).

3.9.3 Fourier Transform Infrared Spectroscopy (FTIR)

FT-IR analysis was conducted to identify the diverse functional groups implicated in the reduction and stabilization of CuO-NPs. The FTIR spectra of copper oxide nanoparticles were obtained using the potassium bromide (KBr) pellet technique, facilitating the evaluation and validation of the functional groups associated with the synthesized nanoparticles. For this purpose, a Schimadzu-Model FTIR instrument located in Kyoto, Japan was utilized. The measurement range was configured within the FTIR spectroscope, employing scan span of 500 to 4000 cm−1and a resolution of 4 cm−1.

3.9.4 Scanning Electron Microscopy (SEM)

The surface characteristics of the biosynthesized NPs were examined through Scanning Electron Microscopy (SEM). The nanoparticles were affixed to stubs covered with a gold palladium layer using double adhesive tape and subsequently subjected to SEM analysis using a JEOL JSM-5910 SEM model situated at the Department of Materials Science & Engineering, Institute of Space Technology, Islamabad. The nanoparticles were coated with gold for four minutes before the SEM examination was conducted at a10 KV accelerating voltage. The images were captured using Polaroid P/N 665 film.

3.9.5 Energy Dispersive X-Ray Spectroscopy (EDS)

EDS, which is generally attached to SEM analysis, was used for the analysis of elemental composition of CuO nanoparticles. The apparatus is installed at the department of materials science & engineering, Institute of Space Technology, Islamabad. Spatially resolved chemical data provided by EDS was used to directly examine the size and morphology of CuO nanoparticles by a high resolution imaging technique based on beam of electrons.

3.9.6 Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) and zeta potential analyses were employed to determine the average particle size and surface charge of the nanoparticles, respectively. The instrument used for this purpose was the Malvern Zeta sizer (Nano ZS90, UK). To perform zeta potential and particle size analyses, the dried M-CuO-NPs powder was suspended in distilled water and subjected to 2 hours of sonication. All measurements were conducted in triplicate, with a temperature stabilization period of 1 minute at 25°C, and at an angle of 90°C.

3.10 Phytochemical Analysis

3.10.1 Total Phenolic Assay

The assessment of TPC involved the utilization of the Folin-Ciocalteu (FC) reagent, following a modified approach based on the technique described by Arias et al. In a 96-well plate, 20 µL of CuO-NPs were taken from each sample with concentrations of 20 µg/mL, 40 µg/mL, 60 µg/mL, and 80 µg/mL. These were mixed with FC reagent (90 µL), which had been previously diluted 10 times using distilled water. Afterward, the mixture was subjected to an incubation at room temperature (25± 2 °C) for five minutes. Following this, 90 µL of Na2CO3 (sodium carbonate) solution (6%, w/v) was introduced to the wells and allowed to incubate for an additional 90 minutes at the same room temperature. For the purpose of comparison, positive control was established using Gallic acid (1 mg/mL), while negative control was set using methanol (20 µL). A UV-Visible spectrophotometer (Shimadzu-1650; Japan) was used to measure the absorbance at 725 nm. The absorbance at 725 nm was measured using a UV-Visible spectrophotometer (Shimadzu-1650; Japan). A calibration curve was generated using 0–40 µg/mL of gallic acid as the standard, resulting in an R2 value of 0.967. The Total Phenolic Content (TPC) was quantified and expressed in terms of GAE/g (DW) [Gallic acid equivalents (GAE) per gram of dry weight (DW)].

3.10.2 Total Flavonoid Assay

The total flavonoid content (TFC) was determined utilizing the AlCl3(aluminum chloride) colorimetric method, following the standard procedure outlined by Xu et al. with slight modifications. To outline the procedure, for each CuO-NPs concentration (20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL), 20 µL of the solution was combined with 10 µL of 10% (w/v) AlCl3 and 10 µL of 1 M potassium acetate. Following this, 160 µL of distilled water was added to the mixture to achieve a total volume of 200 microliters. The resulting mixture was then allowed to incubate

at room temperature (25±2 °C) for a duration of 30 minutes. The absorbance of the resultant mixture was then measured with a UV-Visible spectrophotometer (Shimadzu-1650; Japan) at a wavelength of 415 nm. A calibration curve was established using quercetin (ranging from 0 to 40 µg/mL) as a standard, resulting in a calibration curve with an R2 value of 0.98. The Total Flavonoid Content (TFC) was quantified in terms of quercetin equivalents (QE) per gram of dry weight (DW). Antioxidant assays

3.10.3 DPPH Radical Scavenging Activity

By slightly altering the procedure described by Fazal et al., the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (FRSA) was carried out to test the antioxidant activity. Briefly, 20 mL of CuO nanoparticles from each concentration (20 µg/mL, 40 µg/mL, 60 µg/mL, and 80 µg/mL) were combined with 180 µL of DPPH solution (3.2 mg/100 mL methanol) in different wells of 96-well plates. Subsequently, the plate was protected from light and left to incubate for an hour at room temperature (25± 2 °C). A UV-visible spectrophotometer was used to detect the reaction mixture's absorbance at a wavelength of 517 nm after the allotted incubation time (Shimadzu-1650; Japan). For the purpose of generating the final concentrations for the negative controls, mixtures of ascorbic acid (at concentrations of 10 µg/mL, 5 µg/mL, 40 µg/mL, and 20 µg/mL) and DMSO (20 µL) were combined with DPPH (180 µL). The radical scavenging activity was calculated as a percentage of DPPH discoloration using the equation below.

Free radical scavenging activity %=100×(1−AE/AD), where

AD is the absorbance of the DPPH solution (standard), and AE is the absorbance of the solution after culture extract was added at a certain concentration.

3.10.4 Total Antioxidant Capacity (TAC)

The total antioxidant potential of the CuO nanoparticles was assessed by measuring the total antioxidant activity using the following method. A total of 180µL of reagent solution (containing 28mM sodium phosphate, 4 mM ammonium molybdate, and 0.6 M sulphuric acid) was mixed with 20µL of CuO nanoparticles from each concentration (20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL) in 96 well plates. The mixture was then incubated in a water bath at 95 °C for 90 minutes. After cooling the incubated sample to room temperature, the absorbance at 695 nm was measured

in comparison to a blank. Antioxidant activity was measured in relation to BHT, which served as the reference. Every assay was performed in triplicate.

3.11 Statistical Analysis

Each experimental condition was replicated three times to ensure accuracy and consistency, all conducted under identical conditions. Microsoft Excel was utilized to calculate standard errors and mean values. Graphs illustrating the data were generated using Origin Pro-2018 software.

CHAPTER 4

RESULTS AND DISCUSSION

4. Results and Discussion

The callus culture of *Echinacea purpurea* is utilized for the first time to synthesize CuO-NPs. After mixing both the copper sulphate pentahydrate solution and plant extract, a color change was observed from greenish to dark brown. The mixture was kept in dark overnight, and CuO-NPs were obtained after subsequent centrifugation, oven drying and calcination. The dried powder of CuO-NPs was kept in Eppendorf covered with aluminum foil due to their sensitivity and stored for further analysis.

Figure 4.1: Colour change shows synthesis of CuO-NPs. **A.** Green coloured extract. **B.** Dark brown coloured extract. **C.** Powdered CuO-NPs after incubation and calcination

4.1 Characterization

Characterization was carried out using the following techniques:

Energy dispersive X-ray spectroscopy (EDS), scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) analysis, UV/VIS Spectrophotometry and dynamic light scattering (DLS).

4.1.1 UV/VIS Spectrophotometry

UV-vis spectroscopy is a very useful technique for analyzing nanoparticle formation and the stability of metal nanoparticles in aqueous solution. The UV-visible absorption spectrum of CuO-NPs prepared from copper sulphate is shown below. The copper oxide nanoparticles prepared have displayed an absorption peak between 280 and 300 nm which is assigned to the absorption of CuO-NPs. The highest peak was observed at 284nm. 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 size and also the shape of the nanoparticles. These results are in support of the study conducted by (I. H. Shah et al., 2022). The UV–Vis spectrophotometry depicted absorbance sharp peak at 285 nm confirming the reduction of $CuSO_4$ into CuO-NPs. Another study conducted by (Altikatoglu et al., 2017) also confirms the adsorption peak of CuO-NPs between 280-300nm.

Figure 4.2: UV/VIS spectra showing peak at 284nm indicating the synthesis of CuO-NPs.

4.1.2 X-Ray Diffraction (XRD) Analysis

XRD serves as a widely used analytical tool for characterizing nanomaterial properties. The XRD spectra of the CuO-NPs synthesized through the green method exhibit ten prominent characteristic peaks located at 2θ angles of 32.7°, 37.5°, 54.6°, 62.8°, 69.3°, and 75.5°. These angles correspond to the (110), (111), (022), (113), (220), and (222) crystallographic planes, respectively, confirming that the particles are crystalline. However, other unknown peaks were also seen, which were explained by the presence of plant extract. Using Scherrer's equation **($D = k\lambda/\beta Cos\theta$)**, the average particle size was calculated. The size of CuNPs was determined to be 32.58 nm by the XRD examination. The plant-produced CuO-NPs were clearly identified as having a face-centered cubic structure by the XRD spectrum, which supports the Joint Committee on Powder Diffraction (JCPD) standard, file number 80-1268. CuO-NP formation is further supported by the clearly visible peak seen in the 2θ range of 35–39°, which agrees with other studies. The XRD patterns seen in this work are consistent with results from earlier studies that used CuO-NPs that were green generated from a variety of plant sources, including *Sida acuta* and *Syzygium aromaticum* (Rajesh et al., 2018; Sathiyavimal et al., 2018). Similarly, XRD studies on *Stereum hirsutum* revealed the same XRD pattern with peaks at 43.6°, 50.7°, and 74.45° and corresponding lattice planes [111], [200] and [220] (Eid et al., 2023).

Figure 4.3: XRD pattern of bio-synthesized CuO-NPs.

4.1.3 Fourier-Transform Infrared Spectroscopy (FTIR)

Most plants have reducing and antioxidant properties that aid in reducing precursor salts. In the context of plant extracts derived from *Echinacea purpurea*, it has been established that a range of biomolecules, including phenols, flavonoids, carboxylic acids, terpenoids, tannins, and proteins play dual roles in both the reduction and capping processes of nanoparticles. FTIR analysis was utilized to pinpoint the functional groups accountable for the capping and reduction in size of the CuO nanoparticles synthesized through the green method. Remarkably, conspicuous absorption peaks were detected at 3251 cm^{-1} in the FTIR spectra, corresponding to the -OH groups typically present in alcohols and phenols. These specific peaks were predominantly localized on the surface of the CuO nanostructures. The notable peaks evident at 1044 cm^{-1}, 1204 cm^{-1}, 1365 cm^{-1}, 1516 cm^{-1},1738 cm^{-1} within the spectrum signified the existence of diverse functional groups, including C-N stretching of aromatic amino groups, C-O carboxylic anions, alcoholic O-H stretching and amine N-H stretching . Moreover, distinctive peaks related to CuO nanoparticles were discerned around 593 cm^{-1}.

Comparable outcomes have been recorded in existing literature, where the synthesis of CuO-NPs using leaf extracts of *Calotropis procera* has been explored. Different absorption peaks have been found at specific wavenumbers, including 985.14 cm^{-1}, 1072.79 cm^{-1}, 1379.35 cm^{-1}, 1591.34 cm^{-1}, 2329.58 cm^{-1} and 3210.44 cm^{-1}. These resonances corresponded to various functional groups present in the synthesized nanoparticles, as highlighted in a study conducted by I. H. Shah et al. in 2022. Another study was conducted by (ROHIT et al., 2015) using aqueous root extract of *Desmodium gangeticum*. The spectrum observed showed frequency bands at 3416 cm^{-1}, 2928 cm^{-1}, 1634 cm^{-1}, 1400 cm^{-1} and 1079 cm^{-1} demonstrating the presence of phytoconstituents from

Desmodium gangeticum on the surface of CuO nanoparticles, which may serve as the capping agent.

Figure 4.4: FTIR spectra of biosynthesized CuO-NPs from *Echinacea purpurea*.

4.1.4 Scanning Electron Microscopy (SEM)

The SEM analysis was employed for the morphological assessment of CuO-NPs. The images unveiled that the CuO-NPs synthesized through the green method exhibited a spherical morphology, displaying a well-dispersed distribution devoid of aggregation. The particle size range fell within 40 to 80 nm, aligning closely with the estimated size deduced from XRD analysis. The notable particle agglomeration observed was attributed to electrostatic forces governing the interparticle attraction. These findings harmonize with prior scholarly reports. Similar results were seen in a work by (Suresh et al., 2020), investigating the green production of CuO-NPs using *Cyperus rotundus* and *Cynodon dactylon* grass extracts. The copper oxide nanoparticles in that study showed a uniform and spherical structure. The size and shape of the prepared CuO-NPs are also in conformation with yet another study conducted by (Altikatoglu et al., 2017) where well-disperesd and spherical shaped CuO-NPs synthesized from *Ocimum basilicum* were reported having particle size ranging under 70nm.

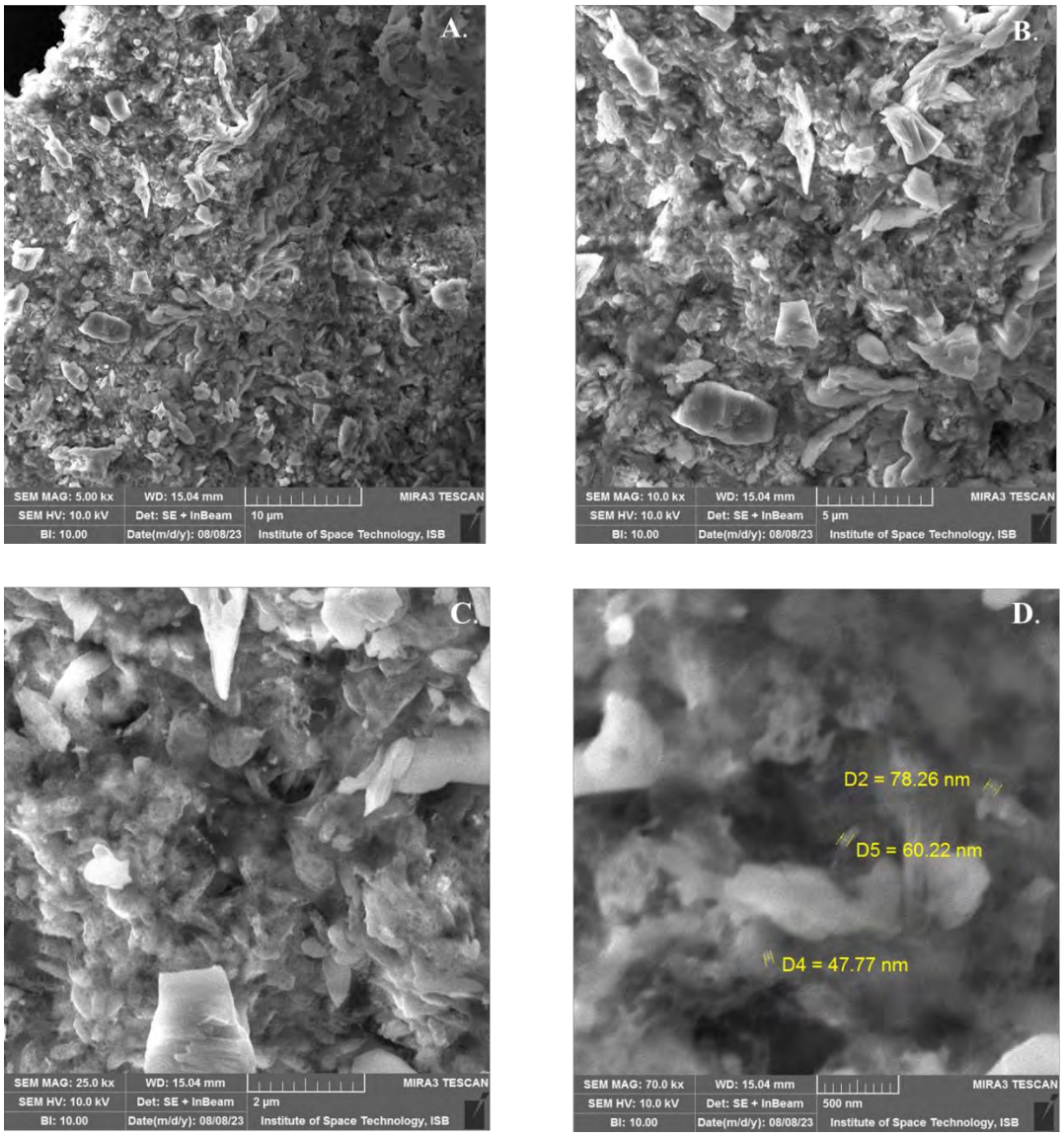


Figure 4.5: SEM images of biosynthesized CuO-NPs under different magnifications. **A.** 10µm. **B.** 5 µm. **C.** 2 µm. **D.** 500nm.

4.1.5 Energy Dispersive X-Ray Spectroscopy (EDS)

EDS analysis shows the elemental composition of the prepared nanoparticles from *Echinacea purpurea.* EDX analysis indicated the presence of oxygen and copper by 36.16% and 35.42%, weight, respectively while other elements like Ca, C, K, P, and S etc. are present in small amounts. The presence of these elements may be as a result of the capping agents present on the surface of CuO nanoparticles. The elemental makeup of the produced nanoparticles was analyzed, disclosing an atomic composition of 55.15% for oxygen and 13.60% for copper. The optical absorption band was examined across the 1 to 9 keV range. Peaks corresponding to binding energies of Cu were

observed at 1 keV and 8 keV, while an additional peak at 0.5 keV indicated the presence of oxygen. The EDS analysis indicated the existence of elemental impurities, consistent with the XRD findings, which could potentially be associated with the presence of enzymes and proteins in the biomass filtrate, and these impurities could be eliminated through elevated temperatures. These outcomes aligned with the research involving CuO-NPs derived from *Mussaenda frondosa,* where the atomic percentages for copper and oxygen were 32.54% and 44.96%, respectively, as reported by (Manasa et al., 2021). Likewise, another study presented an analogous EDS spectrum for copper oxide nanoparticles, with copper and oxygen accounting for 79.96% and 20.04% by weight, respectively, resulting in atomic percentages of 50.12% and 49.88%, as detailed in the work of (Gaba et al., 2022).

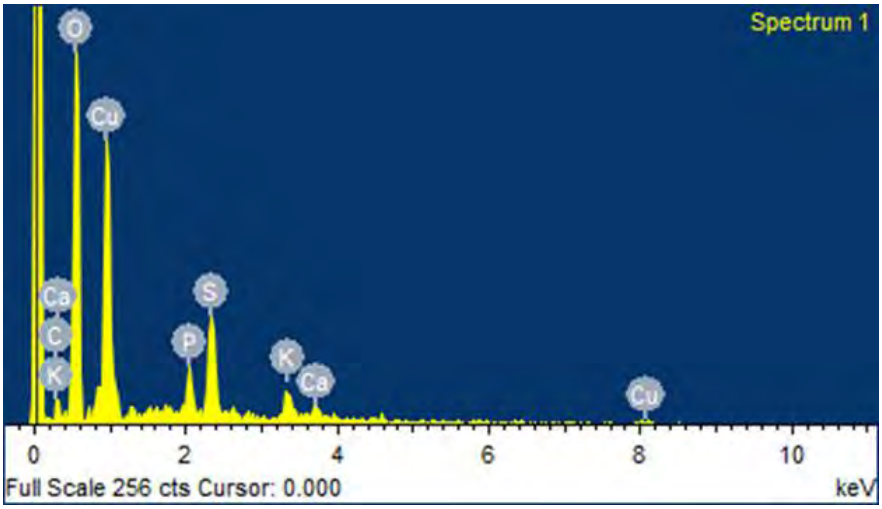


Figure 4.6: EDS spectrum of biosynthesize CuO-NPs showing their elemental makeup.

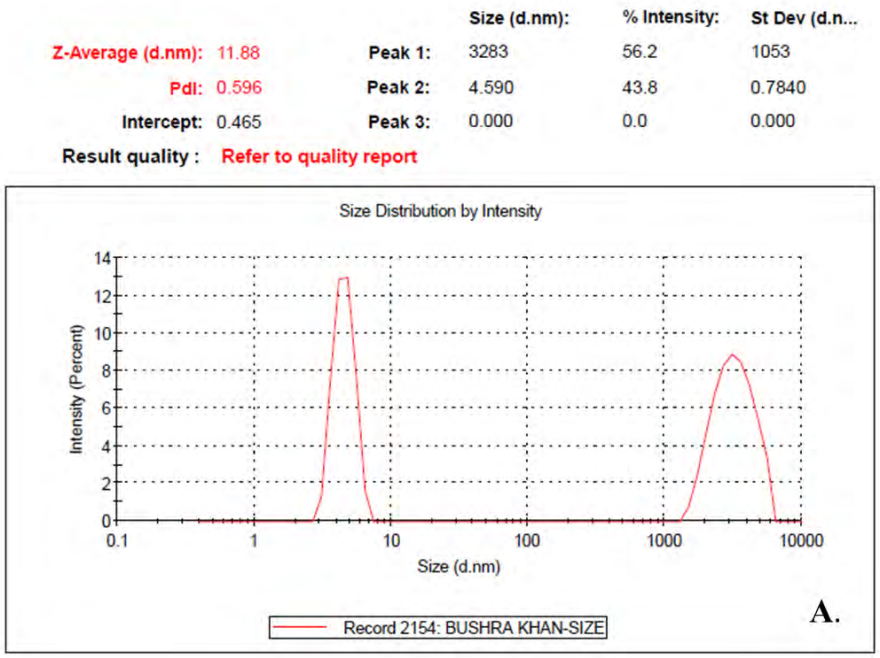
4.1.6 Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) was employed to evaluate the size, distribution, and hydrodynamic properties of CuO-NPs generated using an aqueous extract of *Echinacea purpurea*. The size distribution plot indicated an average particle size of 11.88 nm for the CuO-NPs synthesized through this green approach. Numerous variables, such as the homogeneity percentages of the nanoparticles in the solution (with larger sizes observed when the distribution is non-homogeneous) and the presence of plant metabolites as a coating agent on the NP surface, can affect the determination of the average particle size using DLS analysis. This coating can affect the calculation, underscoring the importance of assessing homogeneity based on the polydispersity index (PDI). A PDI value below 0.4 indicates enhanced homogeneity of NPs in the colloidal

solution, while values exceeding 0.4 signify decreased homogeneity. A PDI greater than 1.0 indicates a heterogeneous distribution. In this case, the PDI value for the callus-based CuO-NPs is 0.5, indicating a homogeneous or similar distribution of NPs within the colloidal solution. These findings are in line with a previous study conducted by (Sarkar et al., 2020), which reported an average diameter of copper oxide nanoparticles as 6.5 ± 1.5 nm, with a particle size range spanning from 1.5 to 20 nm.

The stability assessment of CuO-NPs synthesized using *Echinacea purpurea* extract was conducted using zeta potential analysis, a technique that examines the electrokinetic behavior of the synthesized nanoparticles within a colloidal solution under the influence of an electric field. Nanomaterials are classified as very unstable when their zeta potential is between ±0 and 10 mV, generally stable between ±10 and 20 mV, moderately stable between ±20 and 30 mV, and highly stable when it is greater than ±30 mV according to the stability classification based on zeta potential values. In the case of CuO-NPs biosynthesized from *Echinacea purpurea*, the measured zeta potential is -6.78 mV, indicating a state of relative stability for the synthesized nanoparticles.

This electrokinetic analysis encompasses a broad scale to effectively discern the surface charges on the nanoparticles. Additionally, capping agents like flavonoids and alkaloids that come from the plant extract might be implicated for the stability shown in plant-derived nanoparticles. These agents enhance the electrostatic forces between particles. Similar findings have been documented in existing literature, where CuO-NPs exhibit a zeta potential of -5.60 mV, signifying their negative charge and moderate stability (Keabadile et al., 2020)



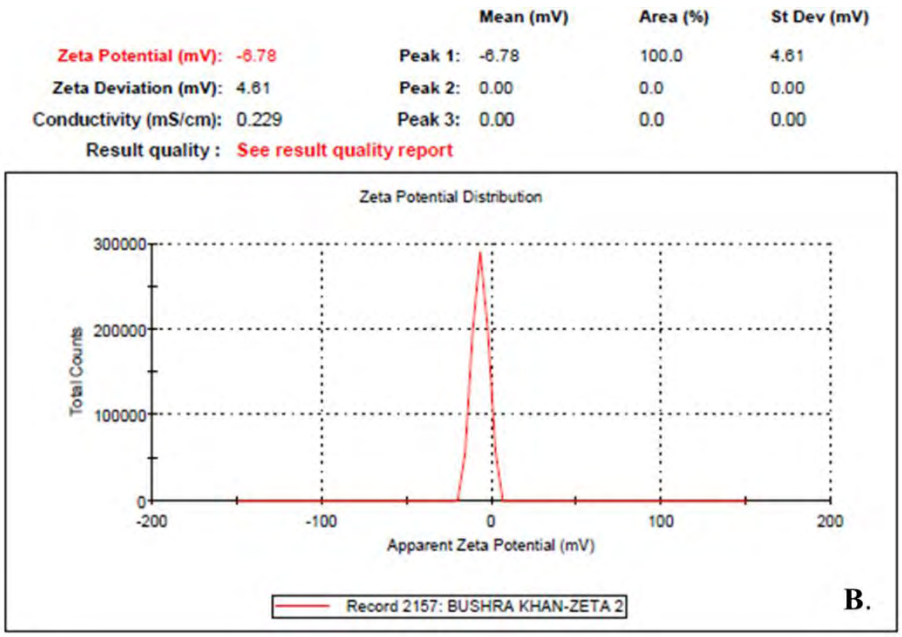


Figure 4.7: DLS analyses of biosynthesized Cu-ONPs. A. zeta size. B. zeta potential.

4.2 Phytochemical Analysis

Phytochemical analysis reveals the existence of several significant phenolic, polyphenolic, and flavonoid compounds in *Echinacea purpurea* callus cultures as reported in literature. These are essential functions of the plant. As a result, its contents in copper oxide NPs derived from callus cultures of *Echinacea purpurea* must be determined.

4.2.1 Total Phenolic Content

Those chemicals are referred to be "phenolics" if they have one or more aromatic rings and one or more hydroxyl groups. Currently, it is known that there are over 8,000 distinct phenolic structures, from straightforward chemicals like phenolic acids to complex polymerized substances like tannins. Over the entire plant kingdom, the secondary metabolites of plants are the ones with the greatest diversity and highest prevalence. Plant phenolics frequently influence a plant's color as well as acting as a defense against UV rays or aggression from pests, pathogens, and predators (Dai & Mumper, 2010). In our study, TPC is studied on copper oxide NPs synthesized through callus cultures of *Echinacea purpurea*. Overall, the highest TPC (24.96 µg GAE/mg DW) was observed on CuO-NPs having highest concentration (80 µg/mL). Whereas the lowest TPC (17.30µg GAE/mg DW) was observed on concentration (20 µg/mL) of CuO nanoparticles. In this study, the value of TPC in invitro grown callus cultures of *Echinacea purpurea* was found to be (16.71 GAE/mg DW) which was significantly comparable to the CuO-NPs synthesized from same plant. Significant amounts of phenol were present in the extract of the in vitro cultivated plant. These potent phytoconstituents have the potential to reduce and stabilize CuO-NPs during green synthesis of CuO-NPs. The inclusion of plant bioactive compounds on the surface of the nanoparticles might increase their biological activity. The TPC values found in our investigation are consistent with research published in the literature. (Nagaraj et al., 2019) have documented the total phenolic content of CuO nanoparticles synthesized using *Pterolobium hexapetalum* leaf extract to be 2(9.63 ± 0.64 µg/mL).

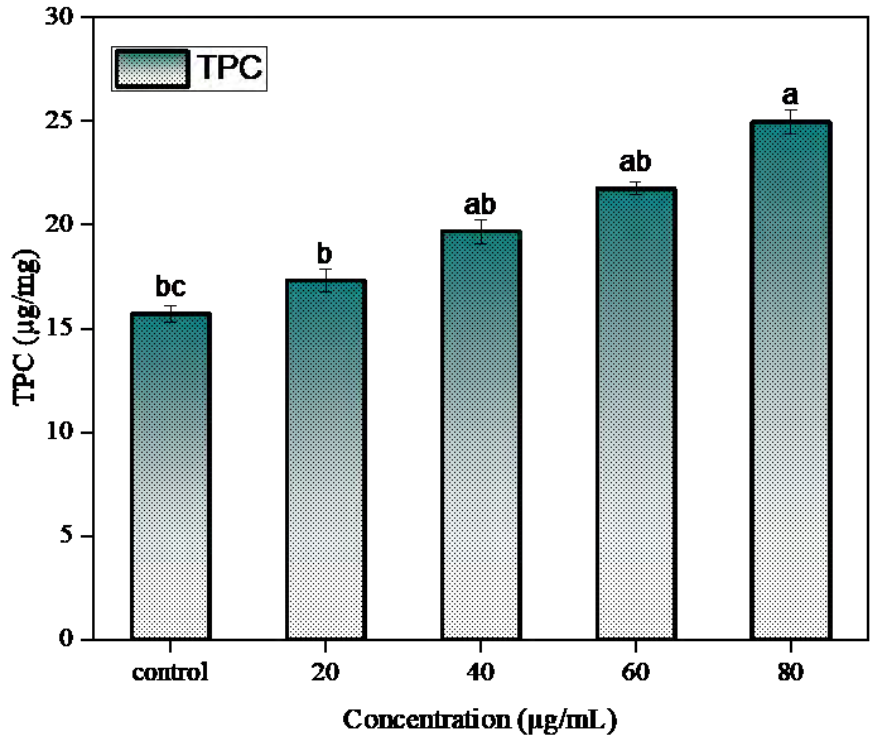


Figure 4.8: Total phenolic content in biosynthesized CuO-NPs under various concentrations.

4.2.2 Total Flavonoid Content

Plants, fruits, and seeds contain enormous amounts of secondary metabolites called flavonoids that give them their color, odor, and flavor. In plants, flavonoids play a number of different activities, including regulating cell growth, attracting insects and pollinators, and protecting against biotic and abiotic stresses (Dias et al., 2021). In the present study, maximum TFC (69.85 µg QE/mg) was observed in CuO-NPs having highest concentration (80 µg/mL), and the lowest TFC (56.39 µg QE/mg) was obtained at extract with lowest concentration (20 µg/mL) of CuO-NPs. In this study, the value of TFC in invitro grown callus cultures of *Echinacea purpurea* was found to be (51.07 µg QE /mg DW) which was significantly comparable to the CuO-NPs synthesized from same plant. Flavonoids contain hydroxyl groups which are responsible for the radical scavenging effect. Studies have reported that CuO-NPs derived from plant sources have similar values of TFC as that of our study. Another study has documented the TFC values for CuO-NPs derived from *Pterolobium hexapetalum* leaf extract to be (68.37 ± 0.64 µg/mL) (Nagaraj et al., 2019).

Figure 4.9: Total flavonoid content in biosynthesized CuO-NPs under various concentrations.

It is discovered that the TPC and TFC results are concentration dependent, meaning that the values of the TPC and TFC increased with increasing concentration of CuO-NPs. Literature has also witnessed the same trend of TPC and TFC values for CuO nanoparticles generated from plant extracts (Zia et al., 2017). The following table depicts the value of TPC and TFC of *Echinacea purpurea* as a control and CuO-NPs with their increasing concentrations.

Table 4.1: Value of TPC and TFC for *Echinacea purpurea* callus extract taken as a control and for CuO-NPs with varying concentrations.

Sample (µg/mL)	TPC (µg/mg)	TFC (µg/mg)
Control (Plant Extract)	16.71139706	51.07051282
CuO-NPs (20)	17.31801471	56.39102564
CuO-NPs (40)	19.67095588	61.19871795
CuO-NPs (60)	21.74816176	65.68589744
CuO-NPs (80)	24.96507353	69.8525641

4.3 Antioxidant Assays

Numerous putative bioactive substances, such as alkaloids, flavonoids, steroids, phenolic acids, and glycosides, have been identified in *Echinacea purpurea*, as documented by (Manasa et al., 2017). Particularly in terms of antioxidant activity, these active ingredients are well-known for their therapeutic effects. The metabolic operation of living things depends critically on antioxidants. Free radicals and reactive oxygen species (ROS) are produced during ordinary physiological processes. These molecules can pose a threat to cells, as they have the potential to oxidize biomolecules, leading to oxidative stress, tissue damage, and cell death. This oxidative stress contributes to the development of various harmful health conditions such as cancer, aging, arthritis, cardiovascular diseases, and neural disorders, among others, as highlighted by (GÜLÇin et al., 2005). Antioxidants serve as essential defenders against the detrimental effects of ROS and free radicals. They assist in maintaining the redox balance within cells, thereby mitigating the potential harm caused by oxidative stress. Given these considerations, it becomes imperative to assess the antioxidant activity of CuO-NPs derived from callus cultures of *Echinacea purpurea*.

The antioxidant capability of copper oxide nanoparticles (NPs) was assessed using DPPH radical scavenging activity and total antioxidant capacity.

4.3.1 DPPH radical scavenging activity

The stability of the DPPH radical arises from the dispersion of an additional electron throughout the molecule, preventing the formation of dimers. The test for DPPH radical scavenging activity assesses the ability of antioxidants to counteract the DPPH radical. Antioxidants facilitate the conversion of DPPH into its nonradical form, leading to the disappearance of its deep purple color. DPPH stands as a persistent nitrogen-centered organic free radical, displaying a rich purple hue that transitions to colorlessness upon reduction to its nonradical state. The DPPH radical model is widely employed to investigate the scavenging capabilities of diverse natural compounds. As the DPPH radical is effectively scavenged, the color of the reaction mixture shifts from purple to yellow, accompanied by a reduction in absorbance at 517 nm. (Ebrahimzadeh et al., 2009)

DPPH radical scavenging activity of *Echinacea purpurea* callus extract and CuO-NPs at different concentrations is depicted in the graph. The CuO-NPs exhibited a significant concentration dependent inhibition of DPPH activity (95.45%) at their highest concentration (80µg/mL) as

compared to *Echinacea purpurea* callus extract (70.6%) which was taken as a control. The lowest value for DPPH activity (84.6%) was recorded against CuO-NPs having lowest concentration (20µg/mL) indicating that this activity is concentration dependent. Literature has reported similar trends of DPPH activity against different concentrations of CuO-NPs synthesized through plant extracts. The % inhibition increased with increasing concentration of CuO-NPs as reported by (Thakar et al., 2022) where the highest DPPH activity (86.78 ± 6.07%) was reported against CuO-NPs having highest concentration (80µg/ml) and vice versa.

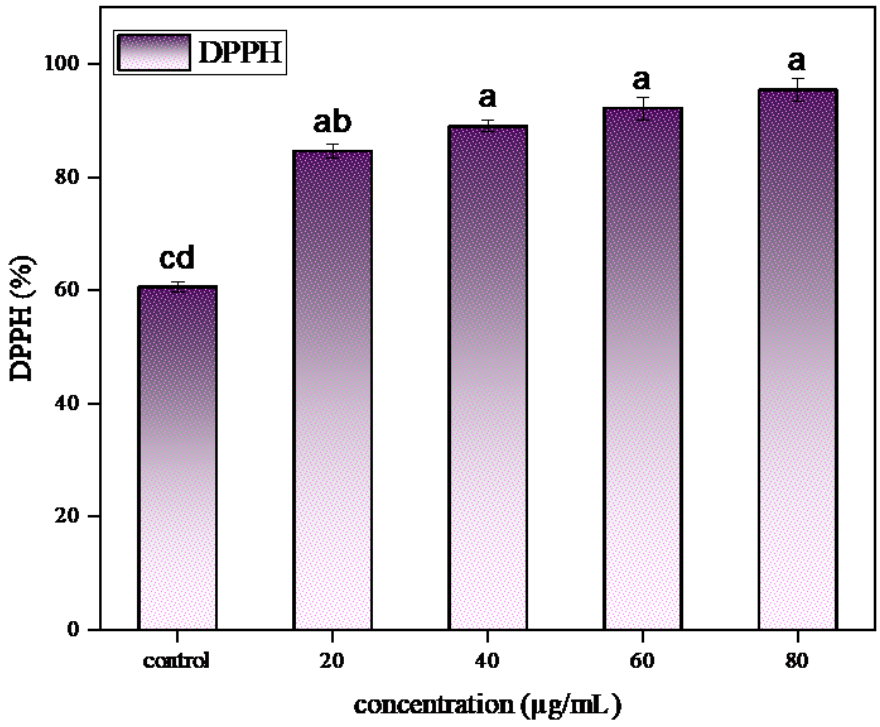


Figure 4.10: DPPH activity of biosynthesized CuO-NPs under various concentrations.

4.3.2 Total Antioxidant Capacity (TAC)

The extract reduces Mo(VI) to Mo(V) and promotes the development of a green phosphate/Mo(V) complex at an acidic pH, which is what accounts for the total antioxidant capacity (TAC). The total antioxidant capacity measures antioxidants that are both fat- and water-soluble. It has been proposed that antioxidant activity is correlated with the electron donating capacity, which reflects the reducing power of bioactive substances. Redox reactions, in which one reactive species is reduced at the expense of the oxidation of the other, can be used to describe the inactivation of oxidants by reductants. Antioxidants play a role of reductant. The presence of reductants, such as

antioxidant substances in CuO-NPs sample, causes the reduction of Mo(VI) to Mo(V) (Aliyu et al., 2013).

Total Antioxidant Capacity of *Echinacea purpurea* callus extract and CuO-NPs at different concentrations is depicted in the graph. According to the data in the graph, the overall antioxidant activity is dosage dependent. At lower concentration of CuO-NPs (20µg/mL), total antioxidant capacity was significantly reduced (12.23µg AAE/mg DW). The CuO-NPs exhibited a significantly higher value of TAC (18.48µg AAE/mg DW) at their highest concentration (80µg/mL) as compared to *Echinacea purpurea* callus extract (10µg/mg) which was taken as a control. The reducing power of the CuO-NPs increased with increasing concentration, which suggests that the electron donating ability of the CuO-NPs is concentration dependent. Similar studies have been conducted and reported in literature where it is shown that TAC has concentration dependent effect on CuO-NPs synthesized through plant extracts. The TAC value increased (49%) with increasing concentration of CuO-NPs (15.0 mM) and decreased (5.9%) with decreasing concentration of NPs (5.0 mM) as reported by (Hassan et al., 2019).

Figure 4.11: Total antioxidant activity of biosynthesized CuO-NPs under various concentrations.

The findings reveal a concentration-dependent relationship between the results of DPPH and TAC assays, indicating that higher concentrations of CuO-NPs correspond to increased values of DPPH and TAC. This trend aligns with observations from existing literature, where similar patterns have been reported for DPPH and TAC values in the case of CuO nanoparticles synthesized from plant extracts (Ijaz et al., 2017)

CHAPTER 5
CONCLUSION

5. Conclusion

This study introduces a highly effective and robust procedure for the facile and environmentally friendly synthesis of CuO nanoparticles (CuO-NPs) through callus-mediated synthesis using *Echinacea purpurea* callus extract. This process offers notable advantages, including its cost-effectiveness. The bio synthesized copper oxide nanoparticles were subjected to a comprehensive characterization process involving UV-Vis spectroscopy, FTIR, SEM, EDS, XRD, and DLS techniques. The FT-IR spectral analysis confirmed the existence of phytochemicals like flavonoids, alkaloids and phenolic compounds within the aqueous extract of *Echinacea purpurea* callus. These findings suggest that these surface-active molecules play a pivotal role in stabilizing the synthesized CuO-NPs by establishing favorable interactions with the surface of CuO. Furthermore, the biosynthesized CuO nanoparticles exhibited notable levels of TFC and TPC, indicating a positive correlation between the concentration of CuO-NPs, the phytochemical composition, and the antioxidant potential. This underscores the potential of these nanoparticles to harness the inherent antioxidant attributes of the plant extract in their synthesis. Notably, the biosynthesized CuO-NPs displayed remarkable antioxidant activity across various antioxidant assays. Given the pivotal role of free radicals in degenerative ailments, the observed antioxidant properties of the biosynthesized CuO-NPs hold promise for advancing novel and potent antioxidants. These nanoparticles also demonstrate the ability to scavenge harmful free radicals, making them potent biocompatible agents suitable for an extensive array of applications in both the biomedical and industrial sectors.

6. References

Abbasi, B. H., Khan, M. A., Mahmood, T., Ahmad, M., Chaudhary, M. F., & Khan, M. A. (2010). Shoot regeneration and free-radical scavenging activity in Silybum marianum L. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *101*, 371–376.

Abid, N., Khan, A. M., Shujait, S., Chaudhary, K., Ikram, M., Imran, M., Haider, J., Khan, M., Khan, Q., & Maqbool, M. (2022). Synthesis of nanomaterials using various top-down and bottom-up approaches, influencing factors, advantages, and disadvantages: A review. *Advances in Colloid and Interface Science*, *300*, 102597.

Afolalu, S. A., Soetan, S. B., Ongbali, S. O., Abioye, A. A., & Oni, A. S. (2019). Morphological characterization and physio-chemical properties of nanoparticle-review. *IOP Conference Series: Materials Science and Engineering*, *640*(1), 12065.

Akintelu, S. A., Folorunso, A. S., Folorunso, F. A., & Oyebamiji, A. K. (2020). Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation. *Heliyon*, *6*(7).

Al-Hakkani, M. F., Gouda, G. A., Hassan, S. H. A., & Nagiub, A. M. (2021). Echinacea purpurea mediated hematite nanoparticles ($α$-HNPs) biofabricatAl-Hakkani, M. F., Gouda, G. A., Hassan, S. H. A., & Nagiub, A. M. (2021). Echinacea purpurea mediated hematite nanoparticles ($α$-HNPs) biofabrication, characterization, physicochem. *Surfaces and Interfaces*, *24*, 101113.

Aliyu, A. B., Ibrahim, M. A., Musa, A. M., Musa, A. O., Kiplimo, J. J., & Oyewale, A. O. (2013). Free radical scavenging and total antioxidant capacity of root extracts of Anchomanes difformis Engl.(Araceae). *Acta Pol Pharm*, *70*(1), 115–121.

Altikatoglu, M., Attar, A., Erci, F., Cristache, C. M., Isildak, I., & others. (2017). Green synthesis of copper oxide nanoparticles using Ocimum basilicum extract and their antibacterial activity. *Fresenius Environ. Bull*, *25*(12), 7832–7837.

Andrievski, R. A. (2014). Review of thermal stability of nanomaterials. *Journal of Materials Science*, *49*, 1449–1460.

Annu, A. A., & Ahmed, S. (2018). Green synthesis of metal, metal oxide nanoparticles, and their

various applications. *Handbook of Ecomaterials*, *2018*, 1–45.

Applerot, G., Lellouche, J., Lipovsky, A., Nitzan, Y., Lubart, R., Gedanken, A., & Banin, E. (2012). Understanding the antibacterial mechanism of CuO nanoparticles: revealing the route of induced oxidative stress. *Small*, *8*(21), 3326–3337.

Arole, V. M., & Munde, S. V. (2014). Fabrication of nanomaterials by top-down and bottom-up approaches-an overview. *J. Mater. Sci*, *1*, 89–93.

Asemani, M., & Anarjan, N. (2019). Green synthesis of copper oxide nanoparticles using Juglans regia leaf extract and assessment of their physico-chemical and biological properties. *Green Processing and Synthesis*, *8*(1), 557–567.

AshaRani, P. V, Low Kah Mun, G., Hande, M. P., & Valiyaveettil, S. (2009). Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano*, *3*(2), 279–290.

Attar, A., & Yapaoz, M. A. (2018). Biomimetic synthesis, characterization and antibacterial efficacy of ZnO and Au nanoparticles using echinacea flower extract precursor. *Materials Research Express*, *5*(5), 55403.

Attarzadeh, M., Balouchi, H., Rajaie, M., Dehnavi, M. M., & Salehi, A. (2020). Improving growth and phenolic compounds of Echinacea purpurea root by integrating biological and chemical resources of phosphorus under water deficit stress. *Industrial Crops and Products*, *154*, 112763.

Awwad, A. M., Albiss, B. A., & Salem, N. M. (2015). Antibacterial activity of synthesized copper oxide nanoparticles using Malva sylvestris leaf extract. *SMU Med J*, *2*(1), 91–101.

Bali, R., & Harris, A. T. (2010). Biogenic synthesis of Au nanoparticles using vascular plants. *Industrial \& Engineering Chemistry Research*, *49*(24), 12762–12772.

Bar, H., Bhui, D. K., Sahoo, G. P., Sarkar, P., De, S. P., & Misra, A. (2009). Green synthesis of silver nanoparticles using latex of Jatropha curcas. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *339*(1–3), 134–139. https://doi.org/10.1016/J.COLSURFA.2009.02.008

Barrett, B. (2003). Medicinal properties of Echinacea: a critical review. *Phytomedicine*, *10*(1),

66–86.

Bayda, S., Adeel, M., Tuccinardi, T., Cordani, M., & Rizzolio, F. (2019). The history of nanoscience and nanotechnology: from chemical--physical applications to nanomedicine. *Molecules*, *25*(1), 112.

Belaeva, T. N., & Butenkova, A. N. (2018). Comparative analysis of the leaf anatomy of Echinacea purpurea and E. pallida. *Biosystems Diversity*, *26*(2), 77–84.

Benelmekki, M. (2015). An introduction to nanoparticles and nanotechnology. In *Designing hybrid nanoparticles*. Morgan \& Claypool Publishers.

Beyene, H. D., Werkneh, A. A., Bezabh, H. K., & Ambaye, T. G. (2017). Synthesis paradigm and applications of silver nanoparticles (AgNPs), a review. *Sustainable Materials and Technologies*, *13*, 18–23.

Billah, M., Hosen, B., Khan, F., & Niaz, K. (2019). *Echinacea Book: Nonvitamin and Nonmineral Nutritional Supplements*. Academic Press: Cambridge, MA, USA.

Biswas, M. C., Chowdhury, A., Hossain, M. M., & Hossain, M. K. (2022). Applications, drawbacks, and future scope of nanoparticle-based polymer composites. In *Nanoparticle-Based Polymer Composites* (pp. 243–275). Elsevier.

Biswas, P., & Wu, C.-Y. (2005). Nanoparticles and the environment. *Journal of the Air \& Waste Management Association*, *55*(6), 708–746.

Bloch, K., Pardesi, K., Satriano, C., & Ghosh, S. (2021). Bacteriogenic platinum nanoparticles for application in nanomedicine. *Frontiers in Chemistry*, *9*, 624344.

Bommakanti, V., Banerjee, M., Shah, D., Manisha, K., Sri, K., & Banerjee, S. (2022). An overview of synthesis, characterization, applications and associated adverse effects of bioactive nanoparticles. *Environmental Research*, 113919.

Bordbar, M., Sharifi-Zarchi, Z., & Khodadadi, B. (2017). Green synthesis of copper oxide nanoparticles/clinoptilolite using Rheum palmatum L. root extract: high catalytic activity for reduction of 4-nitro phenol, rhodamine B, and methylene blue. *Journal of Sol-Gel Science and Technology*, *81*, 724–733.

Brown, P. N., Chan, M., & Betz, J. M. (2010). Optimization and single-laboratory validation study of a high-performance liquid chromatography (HPLC) method for the determination of phenolic Echinacea constituents. *Analytical and Bioanalytical Chemistry*, *397*, 1883–1892.

Buazar, F., Sweidi, S., Badri, M., & Kroushawi, F. (2019). Biofabrication of highly pure copper oxide nanoparticles using wheat seed extract and their catalytic activity: A mechanistic approach. *Green Processing and Synthesis*, *8*(1), 691–702.

Carvalho, C. R., Wedge, D. E., Cantrell, C. L., Silva-Hughes, A. F., Pan, Z., Moraes, R. M., Madoxx, V. L., & Rosa, L. H. (2016). Molecular phylogeny, diversity, and bioprospecting of endophytic fungi associated with wild ethnomedicinal North American plant Echinacea purpurea (Asteraceae). *Chemistry \& Biodiversity*, *13*(7), 918–930.

Catanzaro, M., Corsini, E., Rosini, M., Racchi, M., & Lanni, C. (2018). Immunomodulators inspired by nature: a review on curcumin and echinacea. *Molecules*, *23*(11), 2778.

Cho, G., Park, Y., Hong, Y.-K., & Ha, D.-H. (2019). Ion exchange: an advanced synthetic method for complex nanoparticles. *Nano Convergence*, *6*, 1–17.

Christian, P., der Kammer, F., Baalousha, M., & Hofmann, T. (2008). Nanoparticles: structure, properties, preparation and behaviour in environmental media. *Ecotoxicology*, *17*, 326–343.

Cuenya, B. R. (2010). Synthesis and catalytic properties of metal nanoparticles: Size, shape, support, composition, and oxidation state effects. *Thin Solid Films*, *518*(12), 3127–3150.

Cuenya, B. R., Baeck, S.-H., Jaramillo, T. F., & McFarland, E. W. (2003). Size-and support-dependent electronic and catalytic properties of Au0/Au3+ nanoparticles synthesized from block copolymer micelles. *Journal of the American Chemical Society*, *125*(42), 12928–12934.

Dagher, S., Haik, Y., Ayesh, A. I., & Tit, N. (2014). Synthesis and optical properties of colloidal CuO nanoparticles. *Journal of Luminescence*, *151*, 149–154.

Darroudi, M., Ahmad, M. Bin, Zamiri, R., Zak, A. K., Abdullah, A. H., & Ibrahim, N. A. (2011). Time-dependent effect in green synthesis of silver nanoparticles. *International Journal of Nanomedicine*, 677–681.

Das, D., Nath, B. C., Phukon, P., & Dolui, S. K. (2013). Synthesis and evaluation of antioxidant and antibacterial behavior of CuO nanoparticles. *Colloids and Surfaces B: Biointerfaces*, *101*, 430–433.

de Oliveira, B. G., Santos, L. F. F., Pianetti, G. A., & César, I. C. (2021). A rapid UPLC method for the simultaneous quantitation of caffeic acid derivatives in dried extracts of Echinacea purpurea. *Journal of Chromatographic Science*, *59*(5), 439–444.

Dias, M. C., Pinto, D. C. G. A., & Silva, A. M. S. (2021). Plant flavonoids: Chemical characteristics and biological activity. *Molecules*, *26*(17), 5377.

Din, M. I., & Rehan, R. (2017). Synthesis, characterization, and applications of copper nanoparticles. *Analytical Letters*, *50*(1), 50–62.

Dykes, P. (2015). Increase in skin surface elasticity in normal volunteer subjects following the use of copper oxide impregnated socks. *Skin Research and Technology*, *21*(3), 272–277.

Eastman, J. A., Choi, S. U. S., Li, S., Yu, W., & Thompson, L. J. (2001). Anomalously increased effective thermal conductivities of ethylene glycol-based nanofluids containing copper nanoparticles. *Applied Physics Letters*, *78*(6), 718–720.

Ebrahimzadeh, M., Ehsanifar, S., & Eslami, B. (2009). Sambucus ebulus elburensis fruits: A good source for antioxidants. *Pharmacognosy Magazine*, *5*(19), 213.

Eid, A. M., Fouda, A., Hassan, S. E.-D., Hamza, M. F., Alharbi, N. K., Elkelish, A., Alharthi, A., & Salem, W. M. (2023). Plant-Based Copper Oxide Nanoparticles; Biosynthesis, Characterization, Antibacterial Activity, Tanning Wastewater Treatment, and Heavy Metals Sorption. *Catalysts*, *13*(2), 348.

El-Batal, A. I., El-Sayyad, G. S., Mosallam, F. M., & Fathy, R. M. (2020). Penicillium chrysogenum-mediated mycogenic synthesis of copper oxide nanoparticles using gamma rays for in vitro antimicrobial activity against some plant pathogens. *Journal of Cluster Science*, *31*, 79–90.

Erenler, R., Telci, I., Ulutas, M., Demirtas, I., Gul, F., Elmastas, M., & Kayir, O. (2015). Chemical Constituents, Quantitative Analysis and Antioxidant Activities of E chinacea purpurea (L.) M oench and E chinacea pallida (N utt.) N utt. *Journal of Food Biochemistry*,

39(5), 622–630.

F\ind\ik, F. (2021). Nanomaterials and their Applications. *Periodicals of Engineering and Natural Sciences*.

Fierascu, I. C., Fierascu, I., Baroi, A. M., Ungureanu, C., Ortan, A., Avramescu, S. M., Somoghi, R., Fierascu, R. C., & Dinu-Parvu, C. E. (2022). Phytosynthesis of biological active silver nanoparticles using Echinacea purpurea L. extracts. *Materials*, *15*(20), 7327.

Gaba, S., Rai, A. K., Varma, A., Prasad, R., & Goel, A. (2022). Biocontrol potential of mycogenic copper oxide nanoparticles against Alternaria brassicae. *Frontiers in Chemistry*, *10*, 966396.

Gahlawat, G., & Choudhury, A. R. (2019). A review on the biosynthesis of metal and metal salt nanoparticles by microbes. *RSC Advances*, *9*(23), 12944–12967.

Gericke, M., & Pinches, A. (2006). Biological synthesis of metal nanoparticles. *Hydrometallurgy*, *83*(1–4), 132–140.

Ghaderian, S. M., & Ravandi, A. A. G. (2012). Accumulation of copper and other heavy metals by plants growing on Sarcheshmeh copper mining area, Iran. *Journal of Geochemical Exploration*, *123*, 25–32.

Ghidan, A. Y., Al-Antary, T. M., & Awwad, A. M. (2016). Green synthesis of copper oxide nanoparticles using Punica granatum peels extract: Effect on green peach Aphid. *Environmental Nanotechnology, Monitoring \& Management*, *6*, 95–98.

Grigore, M. E., Biscu, E. R., Holban, A. M., Gestal, M. C., & Grumezescu, A. M. (2016). Methods of synthesis, properties and biomedical applications of CuO nanoparticles. *Pharmaceuticals*, *9*(4), 75.

Gu, Q., Lv, J., Mo, X., & Jiang, X. (2022). High aspect ratio metamaterials and their applications. *Sensors and Actuators A: Physical*, *335*, 113376.

GÜLÇin, I., Alici, H. A., & Cesur, M. (2005). Determination of in vitro antioxidant and radical scavenging activities of propofol. *Chemical and Pharmaceutical Bulletin*, *53*(3), 281–285.

Guo, D., Xie, G., & Luo, J. (2013). Mechanical properties of nanoparticles: basics and

applications. *Journal of Physics D: Applied Physics*, *47*(1), 13001.

Hano, C., & Abbasi, B. H. (2021). Plant-based green synthesis of nanoparticles: Production, characterization and applications. In *Biomolecules* (Vol. 12, Issue 1, p. 31). MDPI.

Hasan, S. (2015). A review on nanoparticles: their synthesis and types. *Res. J. Recent Sci*, *2277*, 2502.

Hassan, S. E.-D., Fouda, A., Radwan, A. A., Salem, S. S., Barghoth, M. G., Awad, M. A., Abdo, A. M., & El-Gamal, M. S. (2019). Endophytic actinomycetes Streptomyces spp mediated biosynthesis of copper oxide nanoparticles as a promising tool for biotechnological applications. *JBIC Journal of Biological Inorganic Chemistry*, *24*, 377–393.

Heiligtag, F. J., & Niederberger, M. (2013). The fascinating world of nanoparticle research. *Materials Today*, *16*(7–8), 262–271.

Hori, H., Teranishi, T., Nakae, Y., Seino, Y., Miyake, M., & Yamada, S. (1999). Anomalous magnetic polarization effect of Pd and Au nano-particles. *Physics Letters A*, *263*(4–6), 406–410.

Hu, M., Butt, H.-J., Landfester, K., Bannwarth, M. B., Wooh, S., & Thérien-Aubin, H. (2019). Shaping the assembly of superparamagnetic nanoparticles. *ACS Nano*, *13*(3), 3015–3022.

Ijaz, F., Shahid, S., Khan, S. A., Ahmad, W., & Zaman, S. (2017). Green synthesis of copper oxide nanoparticles using Abutilon indicum leaf extract: Antimicrobial, antioxidant and photocatalytic dye degradation activitie. *Tropical Journal of Pharmaceutical Research*, *16*(4), 743–753.

Ijaz, I., Gilani, E., Nazir, A., & Bukhari, A. (2020). Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. *Green Chemistry Letters and Reviews*, *13*(3), 223–245.

Jäger, T., Mokos, A., Prasianakis, N. I., & Leyer, S. (2022). first_page settings Order Article Reprints Open AccessArticle Pore-Level Multiphase Simulations of Realistic Distillation Membranes for Water Desalination. *Membranes*.

Jemilugba, O. T., Parani, S., Mavumengwana, V., Oluwafemi, O. S., & others. (2019). Green

synthesis of silver nanoparticles using Combretum erythrophyllum leaves and its antibacterial activities. *Colloid and Interface Science Communications*, *31*, 100191.

Joudeh, N., & Linke, D. (2022). Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. *Journal of Nanobiotechnology*, *20*(1), 262.

Jun, Y., Seo, J., & Cheon, J. (2008). Nanoscaling laws of magnetic nanoparticles and their applicabilities in biomedical sciences. *Accounts of Chemical Research*, *41*(2), 179–189.

Karimi, N., Behbahani, M., Dini, G., & Razmjou, A. (2018). Enhancing the secondary metabolite and anticancer activity of Echinacea purpurea callus extracts by treatment with biosynthesized ZnO nanoparticles. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, *9*(4), 45009.

Katwal, R., Kaur, H., Sharma, G., Naushad, M., & Pathania, D. (2015). Electrochemical synthesized copper oxide nanoparticles for enhanced photocatalytic and antimicrobial activity. *Journal of Industrial and Engineering Chemistry*, *31*, 173–184.

Keabadile, O. P., Aremu, A. O., Elugoke, S. E., & Fayemi, O. E. (2020). Green and traditional synthesis of copper oxide nanoparticles—Comparative study. *Nanomaterials*, *10*(12), 2502.

Kelkawi, A. H. A., Abbasi Kajani, A., & Bordbar, A.-K. (2017). Green synthesis of silver nanoparticles using Mentha pulegium and investigation of their antibacterial, antifungal and anticancer activity. *IET Nanobiotechnology*, *11*(4), 370–376.

Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*, *12*(7), 908–931.

Khan, Y., Sadia, H., Ali Shah, S. Z., Khan, M. N., Shah, A. A., Ullah, N., Ullah, M. F., Bibi, H., Bafakeeh, O. T., Khedher, N. Ben, & others. (2022). Classification, synthetic, and characterization approaches to nanoparticles, and their applications in various fields of nanotechnology: A review. *Catalysts*, *12*(11), 1386.

Khlebtsov, N., & Dykman, L. (2011). Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies. *Chemical Society Reviews*, *40*(3), 1647–1671.

Kindscher, K. (2016). *Echinacea: Herbal medicine with a wild history*. Springer.

Kindscher, K., & Wittenberg, R. (2016). The Naming and Classification of Echinacea Species. *Echinacea: Herbal Medicine with a Wild History*, 37–45.

Kiruba Daniel, S. C. G., Vinothini, G., Subramanian, N., Nehru, K., & Sivakumar, M. (2013). Biosynthesis of Cu, ZVI, and Ag nanoparticles using Dodonaea viscosa extract for antibacterial activity against human pathogens. *Journal of Nanoparticle Research*, *15*, 1–10.

Kolahalam, L. A., Viswanath, I. V. K., Diwakar, B. S., Govindh, B., Reddy, V., & Murthy, Y. L. N. (2019). Review on nanomaterials: Synthesis and applications. *Materials Today: Proceedings*, *18*, 2182–2190.

Kolhatkar, A. G., Jamison, A. C., Litvinov, D., Willson, R. C., & Lee, T. R. (2013). Tuning the magnetic properties of nanoparticles. *International Journal of Molecular Sciences*, *14*(8), 15977–16009.

Kouhkan, M., Ahangar, P., Babaganjeh, L. A., & Allahyari-Devin, M. (2020). Biosynthesis of copper oxide nanoparticles using Lactobacillus casei subsp. casei and its anticancer and antibacterial activities. *Current Nanoscience*, *16*(1), 101–111.

Krishnan, B., & Mahalingam, S. (2017). Improved surface morphology of silver/copper oxide/bentonite nanocomposite using aliphatic ammonium based ionic liquid for enhanced biological activities. *Journal of Molecular Liquids*, *241*, 1044–1058.

Kristianto, H., Prasetyo, S., & Sugih, A. K. (2022). Green synthesized iron nanoparticles using Leucaena leucocephala crude extract as Fenton-like catalyst. *AIP Conference Proceedings*, *2493*(1).

Kuchibhatla, S. V. N. T., Karakoti, A. S., Baer, D. R., Samudrala, S., Engelhard, M. H., Amonette, J. E., Thevuthasan, S., & Seal, S. (2012). Influence of aging and environment on nanoparticle chemistry: implication to confinement effects in nanoceria. *The Journal of Physical Chemistry C*, *116*(26), 14108–14114.

Kumbhakar, P., Ray, S. S., & Stepanov, A. L. (2014). Optical properties of nanoparticles and nanocomposites. *Journal of Nanomaterials*, *2014*, 1.

Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Vander Elst, L., & Muller, R. N. (2008). Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chemical Reviews*, *108*(6), 2064–2110.

Lei, Y.-M., Huang, W.-X., Zhao, M., Chai, Y.-Q., Yuan, R., & Zhuo, Y. (2015). Electrochemiluminescence resonance energy transfer system: mechanism and application in ratiometric aptasensor for lead ion. *Analytical Chemistry*, *87*(15), 7787–7794.

Liu, H., Zheng, S., Xiong, H., Alwahibi, M. S., & Niu, X. (2020). Biosynthesis of copperoxide nanoparticles using Abies spectabilis plant extract and analyzing its antinociceptive and anti-inflammatory potency in various mice models. *Arabian Journal of Chemistry*, *13*(9), 6995–7006.

Liu, Z., Lv, Y., Zhao, N., Guan, G., & Wang, J. (2015). Protein kinase R-like ER kinase and its role in endoplasmic reticulum stress-decided cell fate. *Cell Death \& Disease*, *6*(7), e1822--e1822.

Lu, Y., & Ozcan, S. (2015). Green nanomaterials: On track for a sustainable future. *Nano Today*, *10*(4), 417–420.

Makvandi, P., Gu, J. T., Zare, E. N., Ashtari, B., Moeini, A., Tay, F. R., & Niu, L. (2020). Polymeric and inorganic nanoscopical antimicrobial fillers in dentistry. *Acta Biomaterialia*, *101*, 69–101.

Mali, S. C., Raj, S., & Trivedi, R. (2019). Biosynthesis of copper oxide nanoparticles using Enicostemma axillare (Lam.) leaf extract. *Biochemistry and Biophysics Reports*, *20*, 100699.

Manasa, D. J., Chandrashekar, K. R., & Bhagya, N. (2017). Rapid in vitro callogenesis and phytochemical screening of leaf, stem and leaf callus of Mussaenda frondosa Linn.: a medicinal plant. *Asian J Pharm Clin Res*, *10*(6), 81–86.

Manasa, D. J., Chandrashekar, K. R., Kumar, D. J. M., Niranjana, M., & Navada, K. M. (2021). Mussaenda frondosa L. mediated facile green synthesis of copper oxide nanoparticles--characterization, photocatalytic and their biological investigations. *Arabian Journal of Chemistry*, *14*(6), 103184.

Mekuye, B., & Abera, B. (2023). Nanomaterials: An overview of synthesis, classification, characterization, and applications. *Nano Select*.

Missana, T., & Adell, A. (2000). On the applicability of DLVO theory to the prediction of clay colloids stability. *Journal of Colloid and Interface Science*, *230*(1), 150–156.

Mistr\'\iková, I., & Vaverková, Š. (2007). Morphology and anatomy of Echinacea purpurea, E. angustifolia, E. pallida and Parthenium integrifolium. *Biologia*, *62*(1), 2–5.

Mondal, S., Roy, N., Laskar, R. A., Sk, I., Basu, S., Mandal, D., & Begum, N. A. (2011). Biogenic synthesis of Ag, Au and bimetallic Au/Ag alloy nanoparticles using aqueous extract of mahogany (Swietenia mahogani JACQ.) leaves. *Colloids and Surfaces B: Biointerfaces*, *82*(2), 497–504. https://doi.org/10.1016/J.COLSURFB.2010.10.007

Mudunkotuwa, I. A., Pettibone, J. M., & Grassian, V. H. (2012). Environmental implications of nanoparticle aging in the processing and fate of copper-based nanomaterials. *Environmental Science \& Technology*, *46*(13), 7001–7010.

Mueller, N. C., & Nowack, B. (2008). Exposure modeling of engineered nanoparticles in the environment. *Environmental Science \& Technology*, *42*(12), 4447–4453.

Muthamil Selvan, S., Vijai Anand, K., Govindaraju, K., Tamilselvan, S., Kumar, V. G., Subramanian, K. S., Kannan, M., & Raja, K. (2018). Green synthesis of copper oxide nanoparticles and mosquito larvicidal activity against dengue, zika and chikungunya causing vector Aedes aegypti. *IET Nanobiotechnology*, *12*(8), 1042–1046.

Nagajyothi, P. C., Muthuraman, P., Sreekanth, T. V. M., Kim, D. H., & Shim, J. (2017). Green synthesis: in-vitro anticancer activity of copper oxide nanoparticles against human cervical carcinoma cells. *Arabian Journal of Chemistry*, *10*(2), 215–225.

Nagaraj, E., Karuppannan, K., Shanmugam, P., & Venugopal, S. (2019). Exploration of bio-synthesized copper oxide nanoparticles using Pterolobium hexapetalum leaf extract by photocatalytic activity and biological evaluations. *Journal of Cluster Science*, *30*, 1157–1168.

Narayanan, K. B., & Sakthivel, N. (2011). Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents.

Advances in Colloid and Interface Science, *169*(2), 59–79.

Nations, S., Long, M., Wages, M., Maul, J. D., Theodorakis, C. W., & Cobb, G. P. (2015). Subchronic and chronic developmental effects of copper oxide (CuO) nanoparticles on Xenopus laevis. *Chemosphere*, *135*, 166–174.

Omer, A. M. (2008). Energy, environment and sustainable development. *Renewable and Sustainable Energy Reviews*, *12*(9), 2265–2300.

Ovais, M., Khalil, A. T., Islam, N. U., Ahmad, I., Ayaz, M., Saravanan, M., Shinwari, Z. K., & Mukherjee, S. (2018). Role of plant phytochemicals and microbial enzymes in biosynthesis of metallic nanoparticles. *Applied Microbiology and Biotechnology*, *102*, 6799–6814.

Pandit, C., Roy, A., Ghotekar, S., Khusro, A., Islam, M. N., Emran, T. Bin, Lam, S. E., Khandaker, M. U., & Bradley, D. A. (2022). Biological agents for synthesis of nanoparticles and their applications. *Journal of King Saud University-Science*, *34*(3), 101869.

Parsons, J. G., Peralta-Videa, J. R., & Gardea-Torresdey, J. L. (2007). Chapter 21 Use of plants in biotechnology: Synthesis of metal nanoparticles by inactivated plant tissues, plant extracts, and living plants. *Developments in Environmental Science*, *5*, 463–485. https://doi.org/10.1016/S1474-8177(07)05021-8

Pham, N.-D., Duong, M.-M., Le, M.-V., Hoang, H. A., & others. (2019). Preparation and characterization of antifungal colloidal copper nanoparticles and their antifungal activity against Fusarium oxysporum and Phytophthora capsici. *Comptes Rendus Chimie*, *22*(11–12), 786–793.

Prakash, S., Elavarasan, N., Venkatesan, A., Subashini, K., Sowndharya, M., & Sujatha, V. (2018). Green synthesis of copper oxide nanoparticles and its effective applications in Biginelli reaction, BTB photodegradation and antibacterial activity. *Advanced Powder Technology*, *29*(12), 3315–3326.

Qiu, L., Zhu, N., Feng, Y., Michaelides, E. E., Żyła Gaweł and Jing, D., Zhang, X., Norris, P. M., Markides, C. N., & Mahian, O. (2020). A review of recent advances in thermophysical properties at the nanoscale: From solid state to colloids. *Physics Reports*, *843*, 1–81.

Rafea, M. A., & Roushdy, N. (2008). Determination of the optical band gap for amorphous and nanocrystalline copper oxide thin films prepared by SILAR technique. *Journal of Physics D: Applied Physics*, *42*(1), 15413.

Raha, S., Mallick, R., Basak, S., & Duttaroy, A. K. (2020). Is copper beneficial for COVID-19 patients? *Medical Hypotheses*, *142*, 109814.

Rai, M., & Nguyen, T. A. (2021). *Nanomaterials Recycling*. Elsevier.

Rai, M., & Yadav, A. (2013). Plants as potential synthesiser of precious metal nanoparticles: progress and prospects. *IET Nanobiotechnology*, *7*(3), 117–124. https://doi.org/10.1049/IET-NBT.2012.0031

Rajesh, K. M., Ajitha, B., Reddy, Y. A. K., Suneetha, Y., & Reddy, P. S. (2018). Assisted green synthesis of copper nanoparticles using Syzygium aromaticum bud extract: Physical, optical and antimicrobial properties. *Optik*, *154*, 593–600.

Raju, D., Mehta, U. J., & Hazra, S. (2011). Synthesis of gold nanoparticles by various leaf fractions of Semecarpus anacardium L. tree. *Trees*, *25*, 145–151.

Razavi, M., Salahinejad, E., Fahmy, M., Yazdimamaghani, M., Vashaee, D., Tayebi, L., Basiuk, V. A., & Basiuk, E. V. (2015). *Green Processes for Nanotechnology*.

Regmi, A., Basnet, Y., Bhattarai, S., Gautam, S. K., & others. (2023). Cadmium Sulfide Nanoparticles: Synthesis, Characterization, and Antimicrobial Study. *Journal of Nanomaterials*, *2023*.

Rehana, D., Mahendiran, D., Kumar, R. S., & Rahiman, A. K. (2017). Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. *Biomedicine \& Pharmacotherapy*, *89*, 1067–1077.

ROHIT, G., SHAKILA, B. A., & GINO, A. K. (2015). *Synthesis of copper oxide nanoparticles using Desmodium gangeticum aqueous root extract*.

Sannino, D. (2021). Types and classification of nanomaterials. *Nanotechnology: Trends and Future Applications*, 15–38.

Sarkar, J., Chakraborty, N., Chatterjee, A., Bhattacharjee, A., Dasgupta, D., & Acharya, K.

(2020). Green synthesized copper oxide nanoparticles ameliorate defence and antioxidant enzymes in Lens culinaris. *Nanomaterials*, *10*(2), 312.

Sathiyavimal, S., Vasantharaj, S., Bharathi, D., Saravanan, M., Manikandan, E., Kumar, S. S., & Pugazhendhi, A. (2018). Biogenesis of copper oxide nanoparticles (CuONPs) using Sida acuta and their incorporation over cotton fabrics to prevent the pathogenicity of Gram negative and Gram positive bacteria. *Journal of Photochemistry and Photobiology B: Biology*, *188*, 126–134.

Savage, T., & Rao, A. M. (2004). Thermal properties of nanomaterials and nanocomposites. In *Thermal Conductivity: Theory, Properties, and Applications* (pp. 261–284). Springer.

Senchina, D. S., Martin, A. E., Buss, J. E., & Kohut, M. L. (2010). Effects of Echinacea extracts on macrophage antiviral activities. *Phytotherapy Research*, *24*(6), 810–816.

Shabbir, Z., Sardar, A., Shabbir, A., Abbas, G., Shamshad, S., Khalid, S., Murtaza, G., Dumat, C., Shahid, M., & others. (2020). Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. *Chemosphere*, *259*, 127436.

Shah, I. H., Ashraf, M., Sabir, I. A., Manzoor, M. A., Malik, M. S., Gulzar, S., Ashraf, F., Iqbal, J., Niu, Q., & Zhang, Y. (2022). Green synthesis and Characterization of Copper oxide nanoparticles using Calotropis procera leaf extract and their different biological potentials. *Journal of Molecular Structure*, *1259*, 132696.

Shah, M., Fawcett, D., Sharma, S., Tripathy, S. K., & Poinern, G. E. J. (2015). Green synthesis of metallic nanoparticles via biological entities. *Materials*, *8*(11), 7278–7308.

Shankar, S., & Rhim, J.-W. (2014). Effect of copper salts and reducing agents on characteristics and antimicrobial activity of copper nanoparticles. *Materials Letters*, *132*, 307–311.

Shao, M., Peles, A., & Shoemaker, K. (2011). Electrocatalysis on platinum nanoparticles: particle size effect on oxygen reduction reaction activity. *Nano Letters*, *11*(9), 3714–3719.

Sharifi-Rad, M., Mnayer, D., Morais-Braga, M. F. B., Carneiro, J. N. P., Bezerra, C. F., Coutinho, H. D. M., Salehi, B., Martorell, M., del Mar Contreras, M., Soltani-Nejad, A., & others. (2018). Echinacea plants as antioxidant and antibacterial agents: From traditional medicine to biotechnological applications. *Phytotherapy Research*, *32*(9), 1653–1663.

Shima, P. D., Philip, J., & Raj, B. (2009). Role of microconvection induced by Brownian motion of nanoparticles in the enhanced thermal conductivity of stable nanofluids. *Applied Physics Letters*, *94*(22).

Shin, W.-K., Cho, J., Kannan, A. G., Lee, Y.-S., & Kim, D.-W. (2016). Cross-linked composite gel polymer electrolyte using mesoporous methacrylate-functionalized SiO2 nanoparticles for lithium-ion polymer batteries. *Scientific Reports*, *6*(1), 26332.

Sifri, C. D., Burke, G. H., & Enfield, K. B. (2016). Reduced health care-associated infections in an acute care community hospital using a combination of self-disinfecting copper-impregnated composite hard surfaces and linens. *American Journal of Infection Control*, *44*(12), 1565–1571.

Singh, A., Gautam, A., Singh, B., & others. (2022). Antiviral effects of medicinal plants and their active phytochemical constituents against respiratory diseases and associated biological functions. In *Coronavirus Drug Discovery* (pp. 23–54). Elsevier.

Singh, A., Singh, N. B., Hussain, I., & Singh, H. (2017). Effect of biologically synthesized copper oxide nanoparticles on metabolism and antioxidant activity to the crop plants Solanum lycopersicum and Brassica oleracea var. botrytis. *Journal of Biotechnology*, *262*, 11–27.

Singh, S. C., Mishra, S. K., Srivastava, R. K., & Gopal, R. (2010). Optical properties of selenium quantum dots produced with laser irradiation of water suspended Se nanoparticles. *The Journal of Physical Chemistry C*, *114*(41), 17374–17384.

Sivaraj, R., Rahman, P. K. S. M., Rajiv, P., Salam, H. A., & Venckatesh, R. (2014). Biogenic copper oxide nanoparticles synthesis using Tabernaemontana divaricate leaf extract and its antibacterial activity against urinary tract pathogen. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *133*, 178–181.

Skumryev, V., Stoyanov, S., Zhang, Y., Hadjipanayis, G., Givord, D., & Nogués, J. (2003). Beating the superparamagnetic limit with exchange bias. *Nature*, *423*(6942), 850–853.

Song, S., Miranda, C. J., Braun, L., Meyer, K., Frakes, A. E., Ferraiuolo, L., Likhite, S., Bevan, A. K., Foust, K. D., McConnell, M. J., & others. (2016). Major histocompatibility complex

class I molecules protect motor neurons from astrocyte-induced toxicity in amyotrophic lateral sclerosis. *Nature Medicine*, *22*(4), 397–403.

Soon, S. L., & Crawford, R. I. (2001). Recurrent erythema nodosum associated with Echinacea herbal therapy. *Journal of the American Academy of Dermatology*, *44*(2), 298–299.

Soufi, G. J., & Iravani, S. (2020). Eco-friendly and sustainable synthesis of biocompatible nanomaterials for diagnostic imaging: current challenges and future perspectives. *Green Chemistry*, *22*(9), 2662–2687.

Stanisavljević, I., Stojičević, S., Veličković, D., Veljković, V., & Lazić, M. (2009). Antioxidant and antimicrobial activities of Echinacea (Echinacea purpurea L.) extracts obtained by classical and ultrasound extraction. *Chinese Journal of Chemical Engineering*, *17*(3), 478–483.

Sukumar, S., Rudrasenan, A., & Padmanabhan Nambiar, D. (2020). Green-synthesized rice-shaped copper oxide nanoparticles using Caesalpinia bonducella seed extract and their applications. *ACS Omega*, *5*(2), 1040–1051.

Suresh, S., Ilakiya, R., Kalaiyan, G., Thambidurai, S., Kannan, P., Prabu, K. M., Suresh, N., Jothilakshmi, R., Kumar, S. K., & Kandasamy, M. (2020). Green synthesis of copper oxide nanostructures using Cynodon dactylon and Cyperus rotundus grass extracts for antibacterial applications. *Ceramics International*, *46*(8), 12525–12537.

Syam Sundar, L., & Sharma, K. V. (2008). Thermal conductivity enhancement of nanoparticles in distilled water. *International Journal of Nanoparticles*, *1*(1), 66–77.

Tabar, R. S., Moieni, A., & Monfared, S. R. (2019). Improving biomass and chicoric acid content in hairy roots of Echinacea purpurea L. *Biologia*, *74*, 941–951.

Thakar, M. A., Jha, S. S., Phasinam, K., Manne, R., Qureshi, Y., & Babu, V. V. H. (2022). X ray diffraction (XRD) analysis and evaluation of antioxidant activity of copper oxide nanoparticles synthesized from leaf extract of Cissus vitiginea. *Materials Today: Proceedings*, *51*, 319–324.

Thakkar, K. N., Mhatre, S. S., & Parikh, R. Y. (2010). Biological synthesis of metallic nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, *6*(2), 257–262.

Thampi, V. V. A., Thanka Rajan, S., Anupriya, K., & Subramanian, B. (2015). Functionalization of fabrics with PANI/CuO nanoparticles by precipitation route for anti-bacterial applications. *Journal of Nanoparticle Research*, *17*, 1–12.

Thomsen, M. O., Christensen, L. P., & Grevsen, K. (2018). Harvest strategies for optimization of the content of bioactive alkamides and caffeic acid derivatives in aerial parts and in roots of Echinacea purpurea. *Journal of Agricultural and Food Chemistry*, *66*(44), 11630–11639.

Tripathy, S., Rodrigues, J., & Shimpi, N. G. (2023). Top-down and Bottom-up Approaches for Synthesis of Nanoparticles. *Nanobiomaterials: Perspectives for Medical Applications in the Diagnosis and Treatment of Diseases*, *145*, 92–130.

Tsai, Y.-L., Chiou, S.-Y., Chan, K.-C., Sung, J.-M., & Lin, S.-D. (2012). Caffeic acid derivatives, total phenols, antioxidant and antimutagenic activities of Echinacea purpurea flower extracts. *LWT-Food Science and Technology*, *46*(1), 169–176.

Vaidehi, D., Bhuvaneshwari, V., Bharathi, D., & Sheetal, B. P. (2018). Antibacterial and photocatalytic activity of copper oxide nanoparticles synthesized using Solanum lycopersicum leaf extract. *Materials Research Express*, *5*(8), 85403.

Vaseghi, Z., Nematollahzadeh, A., & Tavakoli, O. (2018). Green methods for the synthesis of metal nanoparticles using biogenic reducing agents: a review. *Reviews in Chemical Engineering*, *34*(4), 529–559.

Verma, N., & Kumar, N. (2019). Synthesis and biomedical applications of copper oxide nanoparticles: an expanding horizon. *ACS Biomaterials Science \& Engineering*, *5*(3), 1170–1188.

Vijayaraghavan, K., & Ashokkumar, T. (2017). Plant-mediated biosynthesis of metallic nanoparticles: A review of literature, factors affecting synthesis, characterization techniques and applications. *Journal of Environmental Chemical Engineering*, *5*(5), 4866–4883.

Waris, A., Din, M., Ali, A., Ali, M., Afridi, S., Baset, A., & Khan, A. U. (2021). A comprehensive review of green synthesis of copper oxide nanoparticles and their diverse biomedical applications. *Inorganic Chemistry Communications*, *123*, 108369.

Wu, Q., Miao, W., Zhang, Y., Gao, H., & Hui, D. (2020). Mechanical properties of

nanomaterials: A review. *Nanotechnology Reviews*, *9*(1), 259–273.

Wu, S. (2007). Preparation of fine copper powder using ascorbic acid as reducing agent and its application in MLCC. *Materials Letters*, *61*(4–5), 1125–1129.

Yugandhar, P., Vasavi, T., Uma Maheswari Devi, P., & Savithramma, N. (2017). Bioinspired green synthesis of copper oxide nanoparticles from Syzygium alternifolium (Wt.) Walp: characterization and evaluation of its synergistic antimicrobial and anticancer activity. *Applied Nanoscience*, *7*, 417–427.

Zhang, P., Tan, W., Zhang, X., Chen, J., Yuan, J., & Deng, J. (2021). Chemical modification of hydroxyl-terminated polybutadiene and its application in composite propellants. *Industrial \& Engineering Chemistry Research*, *60*(10), 3819–3829.

Zharov, V. P., Kim, J.-W., Curiel, D. T., & Everts, M. (2005). Self-assembling nanoclusters in living systems: application for integrated photothermal nanodiagnostics and nanotherapy. *Nanomedicine: Nanotechnology, Biology and Medicine*, *1*(4), 326–345.

Zia, M., Gul, S., Akhtar, J., Haq, I. ul, Abbasi, B. H., Hussain, A., Naz, S., & Chaudhary, M. F. (2017). Green synthesis of silver nanoparticles from grape and tomato juices and evaluation of biological activities. *IET Nanobiotechnology*, *11*(2), 193–199.

beekay

ORIGINALITY REPORT

16%	10%	12%	7%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of Warwick Student Paper	1%
2	www.mdpi.com Internet Source	<1%
3	Submitted to Higher Education Commission Pakistan Student Paper	<1%
4	Bilal Haider Abbasi, Taimoor Khan, Razia Khurshid, Muhammad Nadeem, Samantha Drouet, Christophe Hano. "UV-C mediated accumulation of pharmacologically significant phytochemicals under light regimes in in vitro culture of Fagonia indica (L.)", Scientific Reports, 2021 Publication	<1%
5	link.springer.com Internet Source	<1%
6	Mawuli K. Azameti, Abdul-Wahab M. Imoro. "Nanotechnology: A promising field in	<1%