STM JOURNALS

Emerging Trends in
Chemical Engineering

https://journals.stmjournals.com/etce

ISSN: 2349-4786 (Online)
Volume 12, Issue 1, 2025
January–April
DOI (Journal): 10.37591/ETCE

Review ETCE

Biomedical Applications of Green-Synthesized Cobalt Oxide Nanoparticles

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Abstract

The biomedical potential of cobalt oxide nanoparticles (Co3O4 NPs) synthesized via green methods is gaining recognition due to their biocompatibility and multifunctionality. This article reviews the antibacterial and anticancer properties of Co3O4 NPs synthesized using Delonix regia leaf extract. Characterization techniques confirmed their nanoscale size and uniform morphology. The antibacterial assay against Escherichia coli revealed significant activity, attributed to enhanced permeability and interaction with bacterial membranes. These findings highlight the promise of green-synthesized Co3O4 NPs for biomedical applications, including infection control and targeted cancer therapy. Harnessing nature's resources, advanced green synthesis methods are revolutionizing nanoparticle production, offering eco-friendly and efficient alternatives to conventional chemical and physical techniques. Because of its exceptional physico-chemical characteristics, the green production of cobalt oxide nanoparticles stands out among these. This study highlights the role of Delonix regia leaf extracts in stabilizing cobalt oxide nanoparticle synthesis, utilizing their abundant functional groups. X-ray diffraction showed peaks between 31.42 and 65.28, with the highest intensity at 36.86, whereas UV-visible spectroscopy revealed a peak at 436 nm for the biosynthesized nanoparticles. The particle size, ranging from 15 to 35 nm, confirmed an average size of 20 nm. SEM analysis displayed uniformly spherical and slightly agglomerated particles. Interestingly, Gram-negative Escherichia coli was significantly inhibited by these green-synthesised nanoparticles. This creative method opens the door for long-term developments in nanotechnology while also highlighting the promise of green chemistry.

Keywords: Cobalt oxide nanoparticles, Antibacterial activity, Biocompatibility, Green synthesis, Biomedical applications, Cancer therapy

INTRODUCTION

The late Norio Taniguchi coined the word "Nanotechnology" in 1974 to describe the ability to precisely create materials at the Nanoscale. In essence, the terms "Nanotechnology" and "Bio-nanotechnology" relate to nanoscale materials and processes that are based on biological, biometric, or biologically inspired molecules, as well as Nano technological devices used to monitor and control biological processes, such as in medicine [1]. Considered to be the live embodiment of the nanotechnology concept is biology. The principles of self-assembly sought after by nanotechnologists appear to be used in the assembly of biological structures at the macromolecular and supramolecular scales. These structures, which are mostly protein-based, may be minuscule processes of amazing intricacy and frequently combine exceptional strength and lightness. The so-called F, ATPase enzyme, which uses a proton gradient across the membrane in which it is embedded to create the universal biological energy source adenosine triphosphate (ATP), and the so-called

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Received Date: December 30, 2024
Accepted Date: January 03, 2025
Published Date: January 10, 2025

Citation: Vandana Vishwakarma, Gourav Mishra. Biomedical Applications of Green-Synthesized Cobalt Oxide Nanoparticles. Emerging Trends in Chemical Engineering. 2024; 12(1): 20–29p.

type III secretion system (TTSS), a spherical assembly of needles found on the surface of some pathogenic bacteria that are used to inject poison into their targets, are examples of amazing mechanical devices [2].

Nanoparticles have emerged as critical components in modern medicine, offering innovative solutions for diagnostics and therapeutics. Cobalt oxide nanoparticles (Co_3O_4 NPs), with their magnetic and catalytic properties, are particularly promising. However, their synthesis using conventional methods poses environmental and economic challenges. Green synthesis using plant extracts provides a sustainable alternative, yielding biocompatible nanoparticles suitable for biomedical use. This study examines the antibacterial and anticancer efficacy of Co_3O_4 NPs synthesized via Delonix regia leaf extract. Figure 1 [3].

Introduction to Nanotechnology

- Norio Taniguchi coined the word "nanotechnology" in 1974 to describe the exact production of materials at the nanoscale.
- Nanotechnology and bio-nanotechnology involve nanoscale materials and processes inspired by biological systems, such as self-assembly principles found in macromolecular structures.
- Examples of nanoscale biological mechanisms include ATPase enzymes and the Type III Secretion System used by certain bacteria.

Applications of Nanotechnology

- Nanotechnology enables the creation of materials with dimensions below 100 nm, used in consumer goods, nano-medicine, nano-electronics, and biomaterials.
- Cobalt oxide nanoparticles (Co_3O_4), due to their optical, catalytic, and magnetic properties, are significant in applications like gas sensors, catalysts, solar energy systems, and biological applications, including cancer treatment.

LITERATURE REVIEW

Cobalt

Cobalt is a silvery, bluish-gray metal ore and relatively rare magnetic element with atomic number 27 and molecular weight with 58.933195 u. Cobalt is Cobaltous (II) and cobaltic (III) are the two valence states. Although it is an essential element, cobalt is found in trace amounts in a mammal's body and is often obtained from a diet high in grains and green vegetables. Humans need the essential element cobalt for vitamin B12 (hydroxocobalamin), which catalyzes processes like the synthesis of methionine, the metabolism of purines and folates, and the conversion of succinic acid to methylmalonic acid.

Physical and chemical properties of Cobalt

The main forms of cobalt found in nature are arsenides, oxides, and sulphides. Cobalt is a gray, brittle, ductile, and magnetic metal that is comparatively uncommon. This relatively non-reactive metal does not oxidize in dry or damp air at room temperature.

Although cobalt is stable in air oxygen, heating causes cobaltous (+2) oxide to form at 900°C and mixed oxides below 900°C. Cobalt does not mix with hydrogen or nitrogen when heated, but it does with carbon, phosphorus, and sulfur. Cobalt is a crucial component of permanent magnets since it is the only metal that can increase the saturation magnetization of iron. Because of its special optical, electrical, magnetic, and catalytic properties, cobalt can be used in a variety of nanoelectronic and nanosensor applications [4].

Cobalt Oxide Nanoparticles (Co_3O_4)

Nanotechnology has developed quickly over the past ten years, and as a result, nanomaterials in general and nanoparticles (NPs) in particular are already present in many areas of our lives. At ambient temperatures, this comparatively non-reactive metal does not oxidize in dry or moist air.

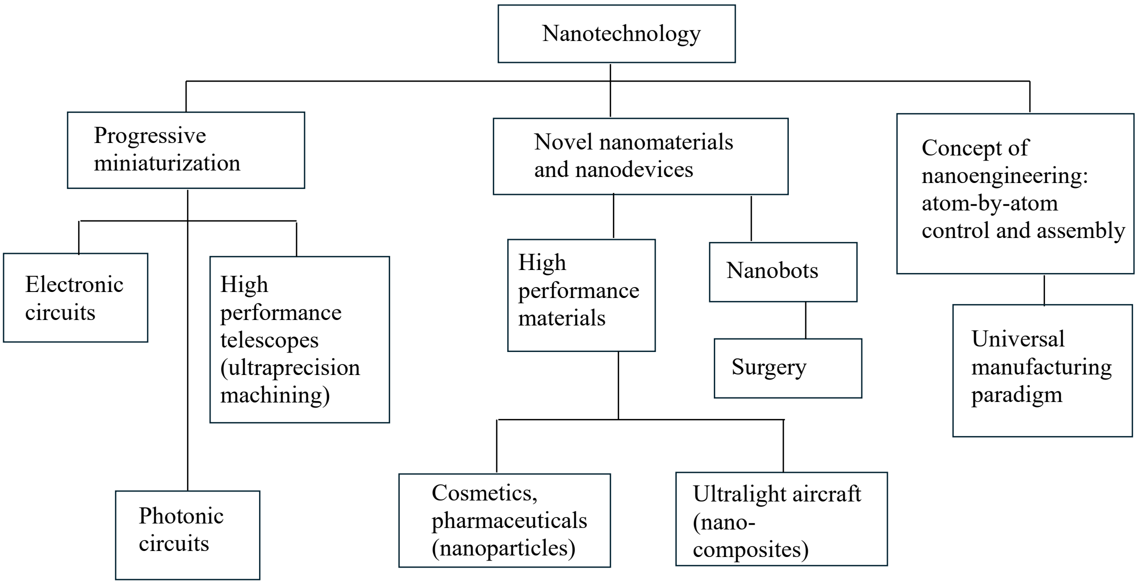

Figure 1. From left to right, the indirect, direct and conceptual branches of nanotechnology, with examples.

In particular, cobalt NPs are destined to find their place in medical biotechnology because of their magnetic properties and due to their distinctive antibacterial, anticancer, catalytic, antioxidant, antifungal, and enzyme inhibitory capabilities, cobalt and cobalt oxide NPs are finding increasing utility in a variety of catalytic and biological applications. Compared to their bulk counterparts, cobalt oxide nanoparticles (Co3O4 NPs) exhibit a wide range of unusual physical and chemical characteristics because of their huge surface area and quantum size effect. Due to its optical, catalytic, and magnetic properties, Co3O4 is a highly significant p-type antiferromagnetic oxide semiconductor with two band gaps. Cobalt oxide nanostructures have a variety of known applications, including anode materials for Li-ion rechargeable batteries, catalysts, gas sensors, supercapacitors, electrochemical sensors, solar absorbers, pH sensors, electrochromic devices, smart windows, and photovoltaic devices. A wide range of forms, including wires, cubes, fibers, tubes, sheets, flowers, and hollow microspheres, have been documented for Co3O4 in the literature. Because the size and shape of the particles may be controlled, it is feasible to alter their properties; as a result, researchers have always tried to minimize the total surface energy [5.]

Biocompatibility of Cobalt Oxide Nanoparticles

Nanomaterials' biocompatibility must be carefully taken into account when using them in biomedical applications. The cobalt phase is always a determining factor in the toxicity of cobalt-containing nanoparticles. The finding that the cobalt oxide phase has more benefits for in vivo applications, higher biocompatibility, and relatively low toxicity when compared to zero-valent cobalt suggests the growing potential of cobalt oxide nanoparticles for biomedical utilization. The biocompatibility of nanoparticles in anti-tumor therapies directly influences the anti-tumor effects, which serves as a reminder that it is crucial to further improve the biocompatibility of cobalt oxide nanoparticles. Although silica and gold nanoparticles are thought to have good biocompatibility, the reticuloendothelial system (RES) can swiftly remove them from the body. By adding marine polysaccharides, several researchers have enhanced the biocompatibility of gold nanoparticles. Similar to this, some active compounds with high biocompatibility may be added to the surface of cobalt oxide nanoparticles to increase their biocompatibility. Cobalt oxide nanoparticles' biocompatibility can also be increased by creating composite materials with other materials. For instance, utilizing silica as the cobalt oxide nanoparticles' protective shell greatly improves their biocompatibility. The cobalt oxide nanoparticles' biocompatibility and bioavailability can also be improved by combining them with other nano preparations. reported. Additionally, the biocompatibility of cobalt oxide nanoparticles produced using various preparation techniques varies. Therefore, making the right decision is crucial [6].

Current Methods for Synthesis of Cobalt Nanoparticles

Since nanoparticles are highly used in many aspects of human life, there is currently a lot of interest in creating novel techniques for generating cobalt nanoparticles. Several essential criteria must be addressed by contemporary techniques to assure the high efficiency of the established process, including:

1. A large production of the intended product
2. The potential to generate nanoparticles with a certain structure (size, shape, and crystallinity)
3. The method's practicality and safety
4. Eco-friendly techniques
5. Expandability

Cobalt nanoparticle preparation methods can be divided into four groups: chemical, biological, physicochemical, and physical. Several tactics should be employed to get beyond the particular challenges that arose throughout the synthesis Figure 2.

Green Techniques for the Synthesis of Cobalt Oxide Nanoparticles

The scientific community has recently been inclined to create a variety of environmentally friendly techniques for the production of materials, especially nanoparticles. These techniques improve the synthetic process's environmental friendliness and safety. Furthermore, because biological systems have a distinct chemical makeup, the nanoparticles produced using these techniques may have distinct physico-chemical characteristics and structures. Due to the capping agent's affinity for compounds found in living organisms (people and bacteria), such nanoparticles also possess higher biocompatibility, which enables the discovery of new systems in which they can be used. One way to create nanoparticles using green chemistry is to reduce cobalt precursors with plant extracts. The plant extracts, which can be either organic or aqueous solvents, serve as reducing and capping agents for the cobalt nanoparticles that are generated. In comparison to nanoparticles produced by a synthetic technique, this process produces unique architectures and good biocompatibility of the resultant nanomaterials. Using extracts from various plants to reduce cobalt precursors is one green chemistry strategy for creating nanoparticles.

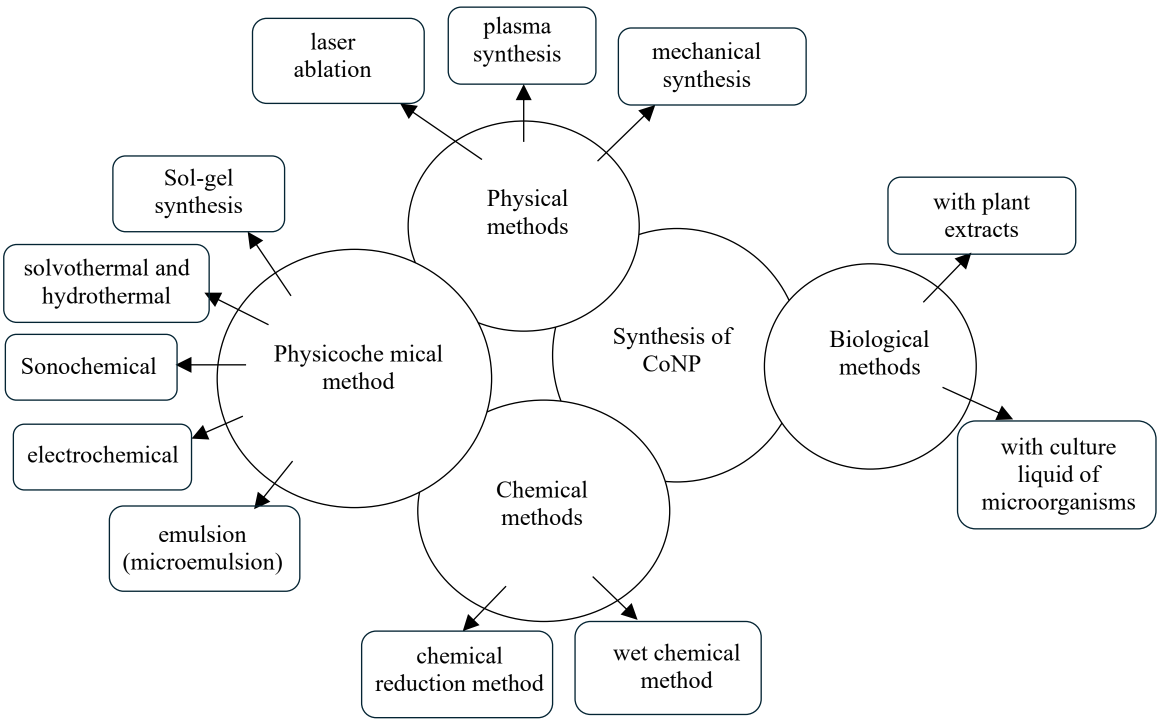

Figure 2 Main methods for the synthesis of cobalt nanoparticles.

The plant extracts serve as reducing and capping agents for the resultant cobalt nanoparticles and can be either organic or aqueous solvents. Compared to nanoparticles made using a synthetic technique, this method yields unique architectures and highly biocompatible nanomaterials because of a collection of extractable chemicals. In contrast to bacteria, algae, and fungi, plants have been extensively used to synthesize cobalt and cobalt oxide NPs. Their quantity, safety, and increased stabilization and decrease of plant phytochemicals are the reasons for this. This method's practicality, commercial feasibility, eco-friendliness, dependability, lack of waste generation, and simplicity have made it a viable alternative to expensive and difficult physicochemical methods. Co and Co3O4 NPs have been synthesized from a variety of plant components, including leaves, roots, stems, fruits, seeds, latex, inner plant parts, shells, and peels [7].

In addition to bacterial cultures, fungi have also been used to manufacture CoNPs, as evidenced by the study of Vijayanandan A. S. and Balakrishnan R. M., in which nanoparticles were made using the fungus Aspergillus nidulans. A green way to make cobalt nanoparticles with Vitis rotundifolia extracts. The basic idea was to add the pulp extract gradually to a cobalt chloride solution while stirring and heating. The resultant system was alkalinized, and a precipitate was separated, dried, and calcined for further use. The size distribution of the resultant nanoparticles was broad. Furthermore, XRD revealed a polycrystalline cubic structure of Co (0) NPs, which might be linked to a variety of compounds in the extract. The mycosynthesised CO_3O_4-NPs using the cell-free filtrate of Aspergillus brasiliensis ATCC 16404. 4 mmol CoSO4·7H2O and 5% filtrate of A. brasiliensis mycelium were cultivated for 72 hours at pH 11 on a shaker in the dark as part of the procedure.During the synthesis, the color of the solution changed from yellow to brown. The mycosynthesized CoNPs were 20–26 nm in size and spherical in form. They demonstrated magnetic characteristics and antibacterial efficacy against a variety of microbes Figure 3 [8].

Antibacterial Activity of Cobalt Nanoparticles

Antibacterial agents are crucial for the textile industry, pharmaceutical sector, food packaging, water disinfection and biomedical areas. Antibiotics are the antibacterial agents that are most frequently used in medical settings. The widespread use and abuse of antibiotics are having a serious negative impact on the development of antibiotic-resistant microorganisms. Infectious diseases that were long under control may now become widespread as a result of this antibiotic resistant strain. Nanoscale materials are one of the new antibacterial substances in use nowadays. The efficacy of these nanomaterials in treating infectious disorders as well as illnesses brought on by antibiotic-resistant bacteria has been demonstrated in both in vivo and in vitro animal models. Gram-negative bacteria P. mirabilis was used in the environmentally friendly synthesis of coupled CoNPs. The characterization of the NPs was confirmed by means of UV-vis, EDX, XRD, TEM, dynamic light scattering (DLS), and polydispersity index (PDI). The antibacterial activity was observed using a well diffusion experiment against Enterococcus faecalis, S. aureus, E. coli, C. perfringens, and S. typhi. The outcome demonstrated that the NPs' bactericidal efficacy against these bacteria is promising.

The fungus species Aspergillus brasiliensis to create quasi-spherical cobalt oxide nanoparticles (NPs) with an average diameter of 20–27 nm. Significant action against several bacteria was demonstrated by the first mycosynthesized cobalt oxide nanoparticles. Omran and associates The disk diffusion method has been used to investigate the antibacterial properties of CoNPs produced by biogenic synthesis utilizing whole plant extract from Celosia argentea. The produced NPs shown exceptional antibacterial properties against E. coli and B. subtilis. Hibiscus rosa-sinensis flower extract was used to create green-mediated cobalt oxide nanoparticles, and their antibacterial efficacy was assessed. These green-synthesised NPs shown encouraging activity against Klebsiella pneumonia, Streptococcus mutans, S. aureus, and E. coli. Two main points have been raised. First, the distinct positive states of cobalt ions, i.e., Co2+ and Co3+, engage with the negatively charged components of the bacterial cell in cobalt oxide nanoparticles and kill the bacterial cell. Second, electrons on the surface of cobalt oxide may be excited by light irradiation in the valence and conduction bands.

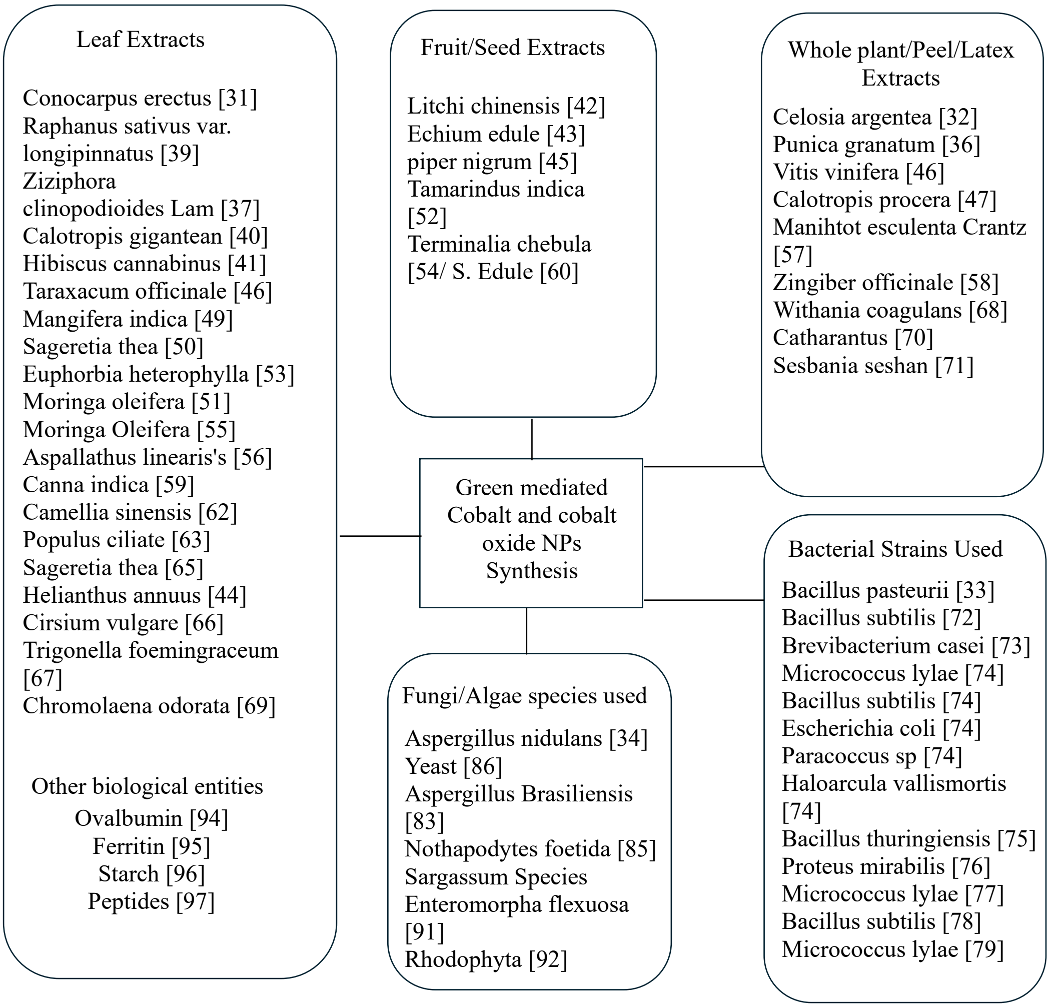

Figure 3. Various methods for the synthesis of cobalt and cobalt oxide NPs.

Because of the interaction between excited electrons and oxygen molecules in the conduction band, superoxide radical anion is formed. Last but not least, hydrogen peroxide, a potent oxidant, is produced. Water and the superoxide radical anion interact to destroy the bacterial cell on the surface of NPs. As a result, at low concentrations, cobalt oxide nanoparticles can have strong antibacterial properties [9].

CoNPs in Catalysis

Different colors are actively catalyzed to break down by cobalt nanoparticles. In order to catalyze the breakdown of methylene orange (MO). The cobalt nanoparticles in the presence of natrium borane. The usage of cobalt nanoparticles and natrium borane together shown high efficacy in the dye's breakdown. The decomposition process was controlled by a spectrophotometric approach, which involved a reduction in the peak at 465 nm's absorption intensity. The dyes get their color from the azo group, and the presence of an azo molecule in the solution is indicated by the peak at 465 nm. This drop in peak intensity and the disappearance of the solution's color are caused by the azo-group being reduced to amino compounds. The suggested process for MO breakdown is the following: CoNPs as a Medicine to Prevent Cancer CoNPs' high surface area, high mass transfer, and magnetic properties make them efficient neoplastic disease therapeutic agents. In aqueous solutions, sodium borohydroxide absorbs on the surface of a cobalt nanoparticle, releasing hydrogen and converting the azo group to amines. The catalytic capabilities of this work are intended to reduce the azo group, which is found in the majority of organic dyes and makes up one- third of the total volume of colours produced. Additionally, because of CoNPs' magnetic characteristics, they can be after usage, detached from the dye recovery solution, regenerate, and reuse. Before being reexamined using TEM and XRD, cobalt nanoparticles were employed as a catalyst four times. The outcome demonstrated that the structure did not change as a result of their use. After the sixth application, however, efficiency significantly dropped while the rate of MO decomposition doubled.

CoNPs as an Anticancer Drug

CoNPs are effective therapeutic agents for neoplastic diseases because of their high surface area, high mass transfer, and magnetic characteristics. They serve as a beneficial delivery system for cytotoxic medications and are also harmful to tumor cells. Research has shown that CoNPs produced with the green method are extremely cytotoxic to cancer cells and show activity against them. CoO/Co3O4 nanoparticles with a cubic spinel shape were created using fresh leaves from young branches of Rosmarinus officinalis. According to XRD data, the optimal concentration of these nanoparticles for cancer therapy is 55 g/mL to U87 cells. Additionally, nanoparticles have magnetic properties that can be exploited for specialised treatments.

In order to assess how CoNPs interacted with bodily biological substances (enzymes, amino acids, etc.), The silico study and suggested a technique for creating nanoparticles that had cytotoxic properties. The XPS approach revealed that the average size of the CoO nanoparticles they created was 41 nm. Furthermore, MTT assay and HCT116 cell apoptosis were carried out, and the results indicated that the IC50 for HCT116 cells was 44 g/mL. Furthermore, it was found that compared to commercial cobalt nanoparticles, green-produced nanoparticles cause lower ROS to trigger apoptosis in colon cells and zebrafish embryos. This technique demonstrates that cobalt nanoparticles can be produced sustainably and used in cancer treatment with little negative effects on the body.

Magnetic Applications of CoNPs

Because of their wide range of morphologies, which differ based on the synthesis method, CoNPs display a diversity of magnetic characteristics. Magnetic CoNPs can be produced in a variety of ways, and they can be applied in many different aspects of daily life. Magnetic CoNPs and items derived from them can also be employed successfully in catalysis.

A way to create CoNPs magnetic nanoparticles, and XRD techniques were used to verify their structure. The authors created nanoparticles with magnetic characteristics and crystal sizes ranging from 2 to 5 nm. Furthermore, because of their core-shell structure, nanosize, and microporous shape, these particles have demonstrated their ability to absorb microwaves. The resulting NPs show promise as a foundation for the development of extremely effective microwave radiation absorbers.

The use of the chemical co-precipitation approach to modify magnetic cobalt nanoparticles with sodium dodecyl sulfate and ligand 2-(5-bromo-2-pyridylazo) -5-diethylaminophenol. The diameter of the cobalt nanoparticles was 100 nm, and this process is inexpensive and easily repeatable. The findings of the study demonstrated that lead ions, which are harmful to the environment, could be effectively selected using these modified nanoparticles. Notably, the resultant nanoparticles may be readily and rapidly extracted from the reaction system because of their magnetic characteristics. The scientists also demonstrated how such systems may be utilized to separate lead ions from other ions, which is useful for quantitatively analyzing the lead ion concentration of different systems. Additionally, it was discovered that these systems had a high degree of regeneration, which lowers process costs and permits cobalt nanoparticle reuse. The superparamagnetic characteristics of these nanomaterials were demonstrated by the modified cobalt nanoparticles' inability to maintain magnetization upon removal of the external magnetic field. Because of their magnetic properties, cobalt nanoparticles can be utilized as an MRI contrast agent. When comparing the produced nanoparticles to iron oxide nanoparticles, they observed that cobalt nanoparticles appear more appealing based on a number of metrics, including relaxing capabilities. Diagnostics employing magnetic resonance techniques will become more effective as a result of this advancement.

According to XRD, Matthias Zeisberger produced magnetic nanoparticles of metallic cobalt Co that were at least 10 nm in size. These nanoparticles outperformed magnetic iron oxide nanoparticles in terms of specific heating power. A particularly promising substance for the diagnosis and treatment of many illnesses is cobalt magnetic nanoparticles. For example, by releasing heat from these particles in an alternating current magnetic field, local hyperthermia can be produced during tumor therapy [10].

Cobalt Oxide Nanoparticles (Co_3O_4)

- These nanoparticles possess unique chemical properties attributed to their quantum size effects and surface area.

Common oxidation states include CoO, Co_2O_3, and Co_3O_4, each with varied applications.

- Using plant extracts, green synthesis offers an economical and sustainable substitute for traditional chemical and physical processes in the production of these nanoparticles.

Project Aim

To biosynthesize cobalt oxide nanoparticles using Delonix regia leaf extract and study their biomedical applications. The aim of the project is to develop an eco-friendly, cost-effective, and efficient method for synthesizing cobalt oxide nanoparticles (Co_3O_4) using Delonix regia leaf extract and explore their biomedical applications. The project emphasizes the green synthesis approach, which reduces the use of harmful chemicals and utilizes bioactive compounds in plant extracts as reducing and capping agents. These nanoparticles are analyzed for their structural, morphological, and functional properties using advanced characterization techniques such as UV-Visible Spectroscopy, FTIR, XRD, TEM, and SEM. Furthermore, the study evaluates the biomedical potential of the synthesized nanoparticles, focusing on their antibacterial activity against Gram-negative bacteria like *E. coli*. The project aims to contribute to the fields of nanotechnology and biomedicine by providing a sustainable and practical method for nanoparticle synthesis, with applications in treating infections and diseases.

OBJECTIVES

1. Synthesize nanoparticles using plant extracts.
2. Characterize the synthesized nanoparticles with UV-Vis spectroscopy, FTIR, XRD, SEM, and TEM.
3. Explore the biomedical applications of cobalt oxide nanoparticles.

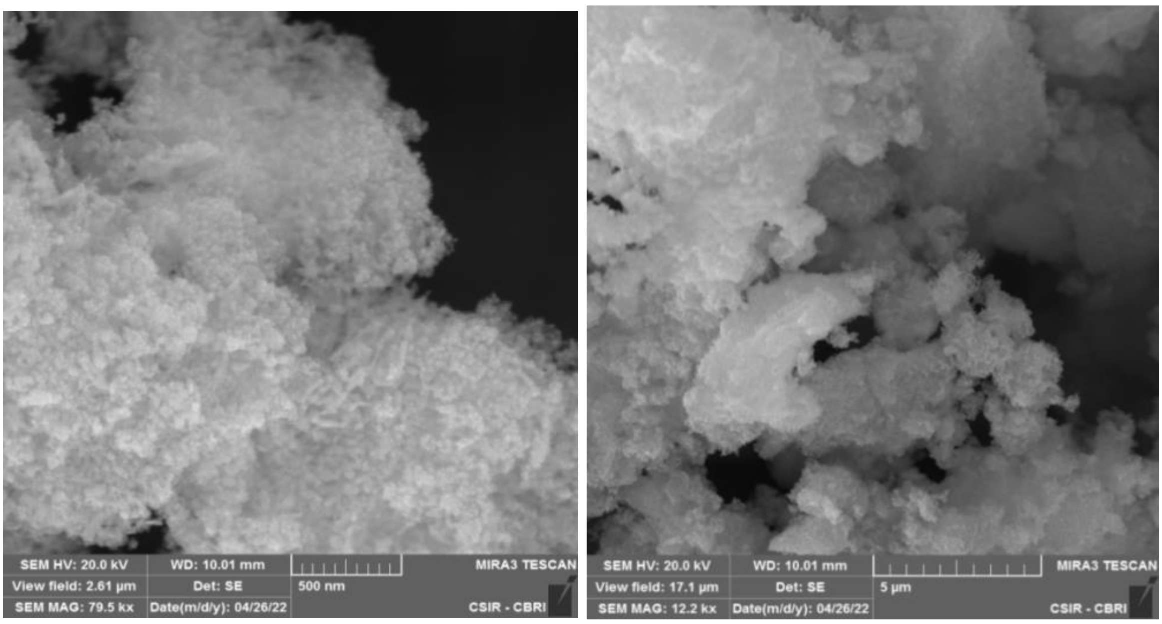

Figure 4. SEM image of green-synthesized Co_3O_4 NPs showcasing uniform size distribution.

Emerging Trends in Chemical Engineering
Volume 12, Issue 1
ISSN: 2349-4786 (Online)

DISCUSSION

The significant antibacterial activity of the green-synthesised Co3O4 NPs was demonstrated by the zone of inhibition in E. coli cultures. The small size and high surface area of the nanoparticles facilitated their interaction with bacterial membranes, disrupting cellular functions and leading to cell death. TEM analysis revealed spherical nanoparticles with a size range of 15–35 nm, while XRD and FTIR confirmed their crystalline structure and functional groups, respectively Figure 4 and 5.

Concentration (mg/ml) | Zone of Inhibition (mm)

Beyond antibacterial properties, Co3O4 NPs exhibit potential in cancer therapy due to their magnetic properties, enabling targeted delivery and localized treatment. The biocompatibility of these nanoparticles, enhanced through green synthesis, reduces toxicity concerns, making them viable for clinical applications. Table 1

Table 1. Physical and Chemical Properties of Cobalt and Its Compounds.

Substance	Molecular formula	Molecular Weight	Melting point	Boiling point	Solubility in Water
Cobalt (II-III) *	Co	58.94	1493°C	3100°C	Limited
Cobaltous oxide (II)*	CoO	74.94	1935°C		0.313 mg/100 g
Cobalt oxides (II- III) *	Co_3O_4	240.80	900°C		Limited
Cobaltic oxide (III)	$Co_2O_3H_2O$	183.88			84 µg/100 g, 37°C
Cobaltous sulfate	$CoSO_4$ $CoSO_47H_2O$	155	96.8°C		39.3 g/100 g, 25°C
Cobaltous chloride	$CoCl_2$	129.84	724°C	1049°C	52.9 g/100 g, 20°C
Cobaltous carbonate	$CoCO_3$	118.94			0.11 g/100 g, 15°C under CO_2 pressure
Cobaltous acetate	$Co(C_2H_3O_2)$	249.08			Soluble

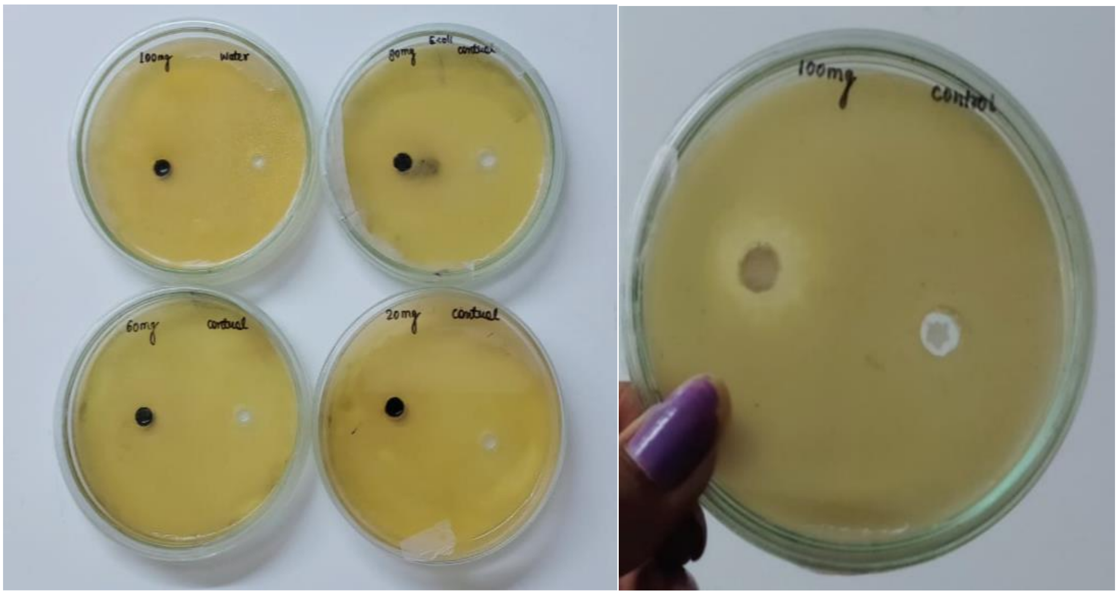

Figure 5. Zone of inhibition observed in E. coli cultures treated with varying concentrations of Co3O4 NPs.

CONCLUSION

The promise of green synthesis techniques in nanotechnology is demonstrated by the thesis on the production of cobalt oxide nanoparticles (Co3O4) using leaf extract from Delonix regia. The study offers a sustainable and eco-friendly substitute for traditional chemical and physical synthesis processes by making use of the natural reducing and stabilizing agents found in plant extracts. With an average size of 20 nm, the produced nanoparticles demonstrated distinct physicochemical characteristics, as demonstrated by characterization methods including UV-Vis spectroscopy, FTIR, SEM, and XRD.

The results demonstrate that the cobalt oxide nanoparticles possess excellent antibacterial properties, particularly against *Escherichia coli*. This can be attributed to their nanoscale size, which enhances interaction with bacterial cell membranes, leading to structural disruption and effective microbial inhibition. The study underscores the viability of using Delonix regia extract as an economical and eco-friendly approach to nanoparticle synthesis, offering a cost-effective solution for biomedical applications.

While the green synthesis process holds promise, challenges such as controlling nanoparticle size and shape remain areas for further research. Future studies should focus on optimizing parameters like pH, temperature, and reactant concentrations to achieve consistent results. Additionally, exploring other plant species for nanoparticle synthesis could expand the range of applications and improve biocompatibility.

This work not only supports the advancement of green chemistry but also lays the groundwork for utilizing biogenic nanoparticles in antibacterial therapies, catalysis, and other industrial applications. It paves the door for nanotechnology's more sustainable future.

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