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Biosynthesis of Iron Nanoparticles from Spinacia Oleracea and its Application in Wastewater Treatment

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Abstract

Water quality and purification issues may be resolved with the aid of "nanotechnology." Due to their prompt responsiveness and effectiveness in eradicating contaminants, along with their capacity to tackle groundwater issues by implementing a permeable reactive barrier utilizing zero-valent iron, zero-valent iron nanoparticles find extensive application in environmental remediation. These nanoparticles, containing zero-valent iron, were produced by synthesizing a metal salt solution with an extract obtained from Spinacia oleracea leaves. In this current study, the plant extract was mixed with a metal salt solution at a predetermined ratio to generate zero-valent iron nanoparticles (FeNP) either at room temperature or via the hydrothermal method. Phase-contrast microscopy and UV-visible spectroscopy were employed to scrutinize the nanoparticles generated. To our knowledge, this study represents the initial exploration of the efficacy of zero-valent iron nanoparticles derived from Spinacia oleracea leaf extract for treating municipal wastewater. The focus is particularly on assessing their impact on BOD and COD parameters.

Keywords: *CPD and BOD reduction, Zero valent iron nanoparticles, UV visible spectra, Phase contrast microscope.*

Introduction

Plants possess the capacity to amass varying levels of heavy metals within specific components of their structures. As a result, the use of plant extracts in biosynthesis processes is growing in popularity as a viable, uncomplicated, efficient, and economical means for nanoparticle production. This approach offers an excellent substitute for conventional preparation methods. Employing a "one-pot" synthesis technique, various plant species can be utilized to stabilize and reduce metallic nanoparticles. To explore a variety of applications for metal/metal oxide nanoparticles, numerous researchers have adopted the eco-friendly synthesis method, crafting particles using extracts obtained from plant leaves[1]. Plant-derived biomolecules, such as proteins, carbohydrates, and coenzymes, possess remarkable capabilities in transforming metal salts into nanoparticles. Initially, investigations into plant extract-assisted synthesis focused on gold and silver metal nanoparticles, mirroring other biosynthesis processes.

The exploration of synthesizing silver and gold nanoparticles has extended to include a diverse range of plant species, such as Tulsi (Ocimum sanctum), aloe vera (Aloe barbadensis Miller), mustard (Brassica juncea), oat (Avena sativa), alfalfa (Medicago sativa), lemon (Citrus limon), coriander (Coriandrum sativum), and lemon grass (Cymbopogon flexuosus). Although the majority of research has focused on the ex vivo production of nanoparticles, there is evidence suggesting that metallic nanoparticles can

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also be formed in vivo within living plants by reducing absorbed metal salt ions in soluble form. The scope of research into silver and gold nanoparticle synthesis has thus expanded to encompass a wide array of plant species. To gain a comprehensive understanding of the plant materials employed in nanoparticle biosynthesis, readers are encouraged to consult Iravani's work.

Rich in numerous nutrients, green leafy vegetables, like spinach, are part of a large group of vegetables that have been called "nature's antiaging wonders" because of their extensive therapeutic usefulness. Within the Chenopodiaceae family is the spinach plant (Spinacia oleracea). Among the most significant vegetables is this one. It's a leafy cool-season vegetable cultivated worldwide, commonly consumed in salads or cooked. Spinach, rich in phytochemicals, possesses bioactive compounds that combat reactive oxygen species, shield against oxidative damage to macromolecules, and influence gene expression and activities tied to metabolism, inflammation, proliferation, and antioxidant defense. Its ability to stimulate the release of satiety hormones contributes to appetite reduction. This research centers on the eco-friendly synthesis of iron oxide nanoparticles, employing spinach as a reducing agent. The study aims to evaluate how these nanoparticles influence Triton X-100-induced processes.

Nanotechnology encompasses the manipulation of substances using chemical and/or physical methods to create materials possessing distinctive properties suitable for various applications. At the core of this discipline lies the nanoparticle (NP), characterized by dimensions less than 100 nm in diameter. NPs are integral in a multitude of fields such as medicine, chemistry, environmental science, energy, agriculture, information technology, communication, heavy industry, and consumer goods, owing to their exceptional optical, thermal, electrical, chemical, and physical attributes. Despite their widespread use, conventional NP synthesis methods such as pyrolysis and attrition have drawbacks, including suboptimal surface formation, limited production rates, and costly, energy-intensive processes. Chemical synthesis techniques, like chemical reduction and the sol-gel method, involve toxic chemicals, potentially leading to hazardous by-products and contamination.

The demand for NP synthesis processes that are safe, free of toxins, and environmentally friendly is on the rise. Biological synthesis protocols offer numerous advantages compared to traditional methods: (i) It represents a clean and eco-friendly approach, eliminating the need for hazardous chemicals. (ii) The active biological component, like an enzyme, plays a dual role by acting as both a reducing and capping agent, leading to reduced synthesis costs. (iii) Large-scale production of small nanoparticles is viable. (iv) Significant energy savings can be achieved by eliminating the requirement for external experimental conditions, such as high energy and pressure. Various biological sources, including plants and microorganisms such as bacteria, yeast, fungi, algae, and viruses, can be utilized for nanoparticle synthesis. [4] The biological production of nanoparticles by plant-mediated means has gained prominence recently, whereas procedures based on microbes have been established through the collaborative efforts of multiple authors. Microbes take longer to remove metal ions than plant extracts do. They are more readily found in nature than microorganisms, plants are the preferred biological resource. Due to their special qualities, which include strong coercivity and superparamagnetism, magnetic nanoparticles (NPs) have gained attention as a new class of significant nanoparticles. These NPs have several disadvantages when produced using conventional techniques. Conventional physics and chemical laws become dormant and inactive at such small scales. Submicrometer iron metal particles are known as iron NPs. NPs' huge surface area makes them extremely reactive [5].

The magnetic characteristics of iron are retained by iron nanoparticles. Owing to their enormous surface area, iron nanoparticles (NPs) are used in targeted medication administration, MRIs, and medical imaging. They also aid in the treatment of a number of neurological illnesses, including Alzheimer's disease. Moreover, organic contaminants in groundwater are scoured using iron nanoparticles. Traditionally, physical and chemical techniques were used to create NPs [6]. The approach of producing iron nanoparticles (NPs) using plant extract is being exploited as a more environmentally friendly method than cultivating microorganism cells. The economical and non-toxic nature of green iron nanoparticle synthesis has garnered interest. This environmentally friendly method, devoid of harmful chemicals, holds promise in addressing various environmental challenges. The study investigates the generation of iron nanoparticles through the utilization of extracts from spinach leaves and banana peels. Known by most as spinach, Spinacia oleracea is a leafy green plant that is a member of the Amaranthaceae family. Because of its abundance of nutrients, spinach is considered a superfood. It has 0.81 g of iron, making it a very good source of iron. It also offers minerals, proteins, and vitamins [7].

Synthesis of Nanoparticles

Nanoparticle production employs two main approaches: top-down and bottom-up methods. The top-down approach explains the nanoparticle creation process by breaking down bulk materials into smaller components. Since the top-down approach uses a large tube furnace to break up bulk material into tiny pieces, it mostly belongs to the physical technique of NP synthesis (Chen et al., 2016). [8-9] Conversely, the bottom-up approach proposes that smaller molecules be used to manufacture NPs in the necessary amount. In this process, chemical reduction is mostly used, sometimes in conjunction with the capping agent for stabilizing the produced NPs. The bottom-up technique also includes the biological process of NP synthesis, in which biomolecules combine with metallic substances to produce nanoscale materials. (figure 1) The concept of bottom-up and top-down tactics. The methods fall into three categories: chemical, biological, and physical methods [10].

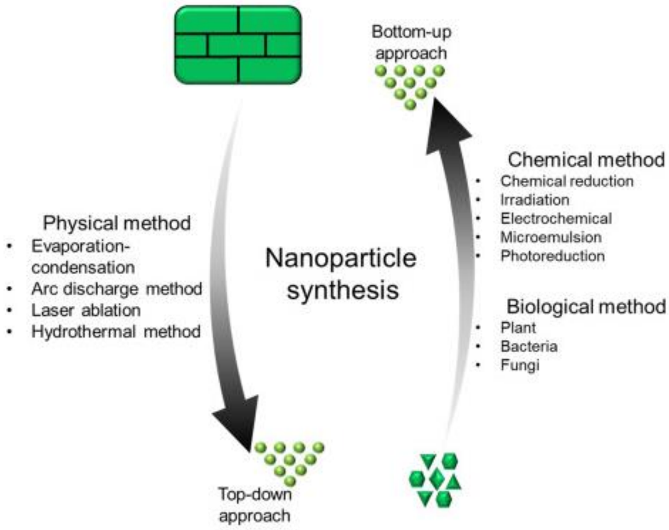


Figure 1: Top-down and bottom-up approach in nanoparticle synthesis

Aims and Objectives

The objectives of the present study include

- To synthesis nanoparticles from the extract of spinach leaves
- To characterize the nanoparticles biosynthesized by silver nanoparticles

Literature Review

In 1991, carbon nanoparticles were first discovered, and by 1993, Iijima and Ichihashi had disclosed the synthesis of single-wall carbon nanotubes measuring one nanometer in diameter (Chen et al., 2021). Often referred to as Bucky tubes, carbon nanotubes are a type of nanomaterial characterized by a hexagonal lattice of carbon atoms arranged in two dimensions. These nanotubes are formed by bending and connecting the carbon atoms in one direction to create a hollow cylindrical structure. The carbon allotropes that fall between graphene (2-dimensional) and fullerene (0-dimensional) are known as carbon nanotubes [11] approximately 120 years prior, Lea documented the creation of citrate-stabilized silver colloid, as reported by Nowack et al. in 2011. This approach results in particles with an average size spanning from 7 to 9 nm. Similar to earlier findings on nanosilver synthesis involving silver nitrate and citrate, this approach achieves nanoscale dimensions and citrate stability. Research dating back to 1902 reveals that proteins were also employed to stabilize nanosilver. The commercially produced nanosilver known as "Collargol" has been in medical use since 1897, boasting a particle size of approximately 10 nanometers (nm). This size characterization was established as early as 1907, confirming Collargol's placement within the nanoscale range. In 1953, Moudry introduced gelatin-stabilized silver nanoparticles, a distinct type of silver nanoparticle, with diameters ranging from 2 to 20 nm. Apart from Collargol, there exists an alternative method for producing these nanoparticles. A patent statement underscores the foresight of nanosilver formulation developers from decades ago, emphasizing the importance of disseminating silver as colloidal particles with a crystallite size less than 25 nm for optimal efficiency [12].

Gold nanoparticles, denoted as AuNPs, boast a rich history in the realm of chemistry, tracing back to the Roman era when they were initially utilized for staining and adorning glassware. The contemporary era of AuNP synthesis commenced over 170 years ago with the pioneering work of Michael Faraday, who may have been the first to observe the distinctive properties exhibited by colloidal gold solutions as opposed to bulk gold. In 1857, Faraday delved into the investigation of the components and production of colloidal suspensions of "Ruby" gold. These nanoparticles, possessing unique optical and electrical attributes, solidify their status as magnetic nanoparticles. Faraday illustrated how gold nanoparticles could yield solutions of varying colors under specific lighting conditions [13].

Materials and Methods

Drugs, Reagents, and Chemicals

The task involved the use of materials such as cardiac marker reagents (Dade Behring/Opus Plus), liver function assay reagents (Randox kits), lipid profile assay reagents (Randox kits), atorvastatin (Gabbyto Pharmacy, Nigeria), Triton X-100, and histopathology reagents. Every chemical and reagent used was of analytical grade.

Plant Material

Figure 2: Hydroponically grown Spinacia oleracea

Fresh spinach, Spinacia oleracea, was collected from farms in Adamawa State, Nigeria, near the Benue River. (figure 2)

Preparation of Plant Sample

Freshly harvested Spinacia oleracea leaves underwent a meticulous cleaning process with running water followed by a rinse with deionized water. After separation from the stem, the leaves were finely ground into powder and allowed to air dry at room temperature. Subsequently, 100 grams of the Spinacia oleracea powder were boiled in 1000 milliliters of double-distilled water in an Erlenmeyer flask, with continuous stirring for 15 minutes to yield the extract. Once cooled to room temperature, the extract underwent filtration using Whatman filter paper number 1.

Green Synthesis of Iron Oxide Nanoparticles

The synthesis of iron oxide nanoparticles involved using Spinacia oleracea leaves and iron chloride as a precursor, following the procedure outlined by Amutha and Sridhar. Initially, a solution containing FeCl3•6H2O and FeCl2•4H2O in a 1:1:2 molar ratio was dissolved in 100 ml of distilled water in a 250 ml beaker. The solution was then heated to 80 °C on a hot plate while gently stirring with a magnetic stirrer. After ten minutes, 20 ml of Spinacia oleracea extract aqueous solution was added, resulting in a noticeable color change from pale yellow to dark brown. To achieve uniform precipitation of sodium hydroxide, 20 ml of an aqueous solution was slowly introduced into the mixture at a rate of 3 ml per minute following an extra 10-minute interval. The mixture is cooled to room temperature, and the resulting iron oxide nanoparticles were isolated through decantation. The produced iron oxide underwent three rinses with distilled water and was subsequently air-dried under room temperature conditions (figure 3).

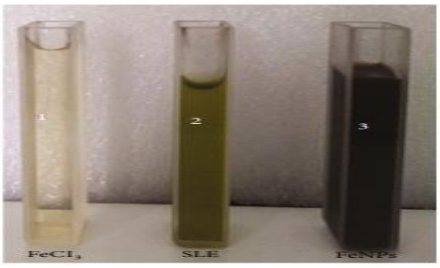


Figure 3: Visual examination of color alterations signifying the creation of iron nanoparticles (FeNPs): spinach leaf extract (SLE***);***

Characterization of Iron Oxide Nanoparticles Produced through the Utilization of Spinacia oleracea Leaf

Size and Structure

By the procedure established by Boukhoubza et al., we employed X-ray diffraction analysis (XRD) to determine the dimensions and composition of the iron oxide nanoparticles generated. After re-dispersing the pellets in sterile double-distilled water and subjecting them to centrifugation at 10,000 rpm for ten minutes, the resulting iron oxide nanoparticles underwent an additional centrifugation step at 10,000 rpm lasting fifteen minutes. The purified pellets were then scrutinized using an XRD (Pan Analytical, X-pert pro, Netherlands) following a baking process in an oven set at 50°C. The iron oxide nanoparticles obtained from Spinacia oleracea leaves were assessed using XRD with a Cu-Kα radiation source, encompassing the 20–80 scattering range on a system operating at 45 kV and 40 mA. X-ray diffraction spectroscopy was utilized to ascertain both the grain size and structure of the synthesized iron oxide nanoparticles.

Composition

FTIR spectroscopy, specifically the IR Prestige21 instrument from Shimadzu Pvt Ltd in Japan, was utilized to investigate the chemical composition of iron oxide nanoparticles. The examination involved employing the KBr pellet technique. This technique involved grinding 1/8 of the solid sample (iron oxide nanoparticles) with 0.50 teaspoons of KBr in a mortar using a pestle, following the procedure outlined by Kero et al.

Results

The Figure 4 displays the FTIR spectrum of the leaf extract derived from Spinacia oleracea. In the FTIR spectrum, distinct peaks are observed at 3335 cm−1, 2110 cm−1, and 1640 cm−1, suggesting the presence of secondary metabolites, alkyne terminal, alkenyl stretching frequency, and the OH stretching vibration associated with the hydroxyl group present in the polyphenols within the extract.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Aqueous Leaf Extract of Spinacia oleracea

XRD Analysis

XRD illustrated in Figure 4 reveals that the iron nanoparticles (FeNPs) produced exhibit an amorphous character. The subtle iron peak observed suggests a noncrystalline structure. In the case of FeNPs obtained from banana peel extract (BPE), distinct peaks at Bragg reflection 2θ = 18.15°, 24.64°, and 35.78° were assigned to crystal planes (110), (200), and (112), respectively. In contrast, FeNPs derived from solvent leaf extract (SLE) exhibited distinct peaks at the Bragg reflection angle of 2θ = 47.55°, predominantly linked to a specific crystal plane, namely (112), along with several combined crystal planes. The utilization of Miller indices confirmed the presence of FeNPs, and the application of Scherrer's formula resulted in a calculated crystallite size of 2.44 nm (figure 4). The XRD pattern revealed unidentified peaks, suggesting the potential crystallization of the bionatural phase from the extract. The presence of these unclassified peaks suggests that certain components from banana peel extracts were incorporated into the produced iron nanoparticles, serving as stabilizing and capping agents to impede the oxidation of iron. When pure Fe3O4 with a spinal structure is made using Tridax procumbens leaf extract, the XRD results are fairly similar. Recent publications also indicated that FeNPs generated by reduction with extracts of sorghum bran and green tea were amorphous.

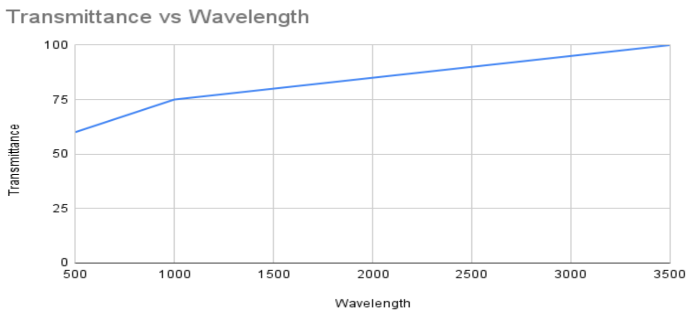
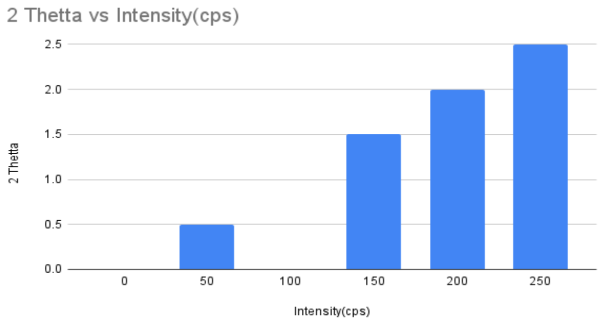


Figure 4: FTIR spectrum of Spinacea oleracea leaf extract

Iron nanoparticles are made using an extract from the leaves of Spinacia oleracea. The reduction of Fe+3 ions into Fe0 nanoparticles occurred when a 2:3 ratio of plant extract to FeCl3 solution was used (figure. 5). The solution's pH changes instantly to black from brown as a result of the reduction of Fe+3 into Fe0. When plant extract was added to an aqueous FeCl3 solution, ferric chloride, which has a strong yellowish color in distilled water, instantly changed the solution's tone and lowered its pH, which was a sign that iron nanoparticles were starting to form. We discovered the pH shift from highly acidic to slightly acidic. Numerous investigations have noted these kinds of outcomes in their investigations. The earlier data indicates a similar outcome, despite the utilization of eucalyptus leaf extract in their study rather than Spinacia oleracea leaves for treating eutrophic wastewater.



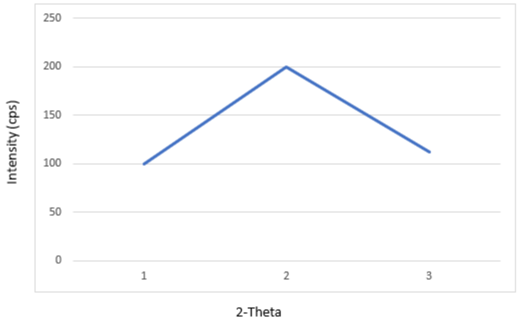


Figure 5: a) Thetta vs intensity graph b) XRD spectrum of biological synthesized SLE-FeNPs

Application of FeNPs in Wastewater Treatment

Highlighting the significance of treating municipal wastewater before discharging it into natural water sources, it is observed that incorporating zero-valent iron nanoparticles in sewage treatment leads to a lowering of BOD levels from an initial 56 mg/L to 22 mg/L, along with a reduction in COD from 405 mg/L to 85 mg/L. Consequently, the calculated removal efficiency for COD is 73.82%, and for BOD, it is 60.31% after a 15-day period, aligning with previously observed removal rates(figure 6). In a study by Suhendrayatna et al. (2012) investigating the photo-reduction process using spontaneous saccharin for municipal wastewater treatment, reported removal efficiencies for BOD and COD are 50.15% and 56.72%, respectively.

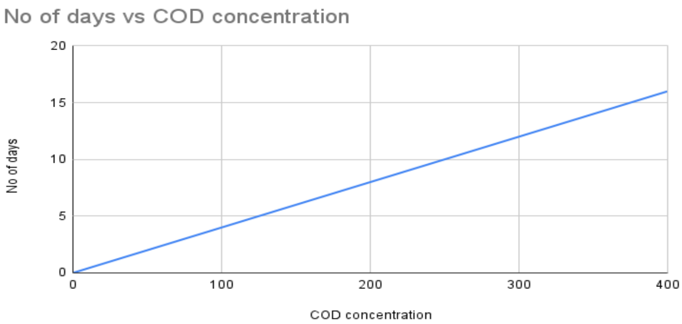


Figure 6: COD reduction from Municipal Waste Water by FeNP from Spinacia oleracea leaves extract

Conclusion

The latest study showcases successful elimination of BOD and COD from municipal wastewater within specified limitations. To ensure the sustainability of nanobiotechnology, it is recommended to adopt an environmentally friendly synthetic approach for synthesizing nanoparticles. By using Spinacia oleracea, zero-valent iron nanoparticles can be generated, demonstrating a capability to decrease COD by 73.82% and BOD by 60.314%. This inventive green chemistry possesses several attractive features, providing a cost-effective and efficient method for environmental protection through bioremediation.

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