



Green Synthesis of Cr₂O₃ NPs Using the Aervalanata Plant Demonstrates Significant Photophysical and Antibacterial Properties

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ABSTRACT:

This research focuses on the eco-friendly synthesis of chromium oxide (Cr₂O₃) nanoparticles utilizing a plant extract from Aervalanata as both a reducing and stabilizing agent. Various characterization methods, including ultraviolet-visible (UV-VIS) spectroscopy and scanning electron microscopy (SEM), were employed to analyze the produced nanoparticles. The nanoparticle sizes averaged between 27 and 40 nm. The antibacterial effectiveness of the green synthesized nanoparticles was assessed against six bacterial strains: *Bacillus subtilis* (BS), *Bacillus cereus* (BC), *Staphylococcus albus* (SA), *Pseudomonas aeruginosa* (PA), *E. coli*, and *Klebsiella pneumoniae* (KP), using agar well diffusion and a live/dead staining assay. The results demonstrated that a concentration of 9 µg/ml of the green Cr₂O₃ NPs displayed significant efficacy against these bacterial strains, which can be attributed to the presence of heterocyclic compounds derived from the plant. The findings indicated that the green synthesized Cr₂O₃ nanoparticles exhibited enhanced antibacterial properties, evidenced by the zones of inhibition (ZOIs) against both Gram-positive and Gram-negative bacteria when compared to the standard antibiotic streptomycin. These observations imply that the enhanced biological activity of the green synthesized Cr₂O₃ nanoparticles may result from a synergistic effect. Therefore, these environmentally friendly Cr₂O₃ nanoparticles could serve as promising candidates for prospective biomedical applications. The findings imply that the enhanced biological activity of the green synthesized Cr₂O₃ nanoparticles may result from a synergistic effect. Consequently, green synthesized Cr₂O₃ nanoparticles could serve as promising candidates for future biomedical applications.

Keywords: Green synthesis, Cr₂O₃, Aervalanata, antibacterial, Nanoparticle, biocompatibility

1. Introduction :

Medicinal plants have long been a cornerstone in the treatment of human ailments, dating back to ancient civilizations. Herbal medicines stand out as a highly effective alternative to modern synthetic drugs, typically exhibiting minimal or no side effects while being regarded as safe options [1-5]. The pursuit of substances with strong antimicrobial properties has become a critical area of research, aimed at significantly reducing the risk of infectious diseases caused by pathogenic bacteria, fungi, viruses, and parasites [6-9]. Plant extracts remain vital sources of numerous therapeutic agents, including powerful antimicrobials for infection treatment. Globally, the application of medicinal plants in managing both acute and chronic wounds is a well-established practice in traditional medicine. One notable example is Aervalanata, a common weed that flourishes throughout the plains of India and is distinguished by its camphor-like aroma [10-12]. The dried flowers, resembling soft spikes, are marketed under the names Buikallan and Boor. Virtually the entire plant is edible, with the leaves commonly incorporated into soups or consumed as spinach or a vegetable. Moreover, Aervalanata is firmly established in traditional medicine for treating snakebites, and the juice extracted from crushed Aervalanata roots is effectively used in the therapy for jaundice. The versatility and efficacy of this plant underscore the value of herbal remedies in contemporary healthcare [13-17].

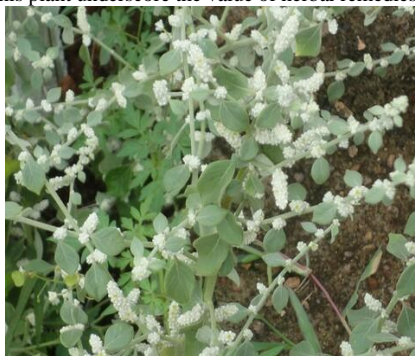


Figure 1. Image showing the Aervalanata plant

Transition metal complex and or their nanoparticles are recognized for their wide range of applications, primarily due to their ability to exist in multiple oxidation states and their large surface areas [18-24]. These characteristics enhance their reactivity compared to bulk materials, making them a significant area of research. One notable compound in this context is chromium trioxide, which has the chemical formula Cr_2O_3 . This red, odorless powder is commonly used alongside additives that influence the plating process without reacting with the chromium trioxide itself [25,26]. In aqueous solutions, chromium trioxide can form chromic acid and various anhydrides. Recently, chromium oxides have garnered substantial interest due to their relevance in both scientific and technological advancements. With chromium's capability to adopt different stable oxidation states, it can generate various types of oxides. Notably, chromium nanoparticles have found numerous applications in the biomedical field, particularly in antibody-related therapies for various diseases [27]. Research indicates that biogenic nanoparticles, which are derived from natural sources, exhibit superior antimicrobial activity compared to their chemically synthesized counterparts. This enhanced property is often attributed to the action of proteins that act as capping agents during the synthesis process [28-33]. For instance, nanoparticles synthesized from the *Aervalanata* plant have proven effective against several bacterial strains, including *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium*, as well as certain pathogenic yeasts.

The pursuit of substances with significant antimicrobial properties has emerged as one of the most active areas of research aimed at reducing the risk of infectious diseases caused by bacteria, fungi, viruses, and parasites that are pathogenic to humans. Plant extracts continue to serve as principal sources for a variety of therapeutic agents, including those utilized in the treatment of infectious diseases [34-38]. The application of medicinal plants in the synthesis of MRI contrast agents for both acute and chronic wounds is prevalent in numerous traditional medicine practices worldwide [39-41]. In this context, numerous plants from tropical and subtropical regions have been assessed for their wound-healing capabilities. Consequently, plants exhibiting antimicrobial activity against multidrug-resistant (MDR) pathogens represent valuable resources [42]. This study focused on the evaluation of the aqueous extract of the medicinal plant *Aervalanata*, followed by the synthesis of metal oxide nanoparticles, examining their absorption characteristics and antimicrobial efficacy against *Bacillus subtilis* (BS), *Bacillus cereus* (BC), *Staphylococcus albus* (SA), *Pseudomonas aeruginosa* (PA), *Escherichia coli*, and *Klebsiella pneumoniae* (KP). Given the bioactive properties of the selected plants and metals, the green-mediated nanoparticles demonstrate potential medicinal value in treating various infections, particularly within traditional medicine frameworks.

2. Materials :

Chromium(III) nitrate nonahydrate [$\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$] and sodium hydroxide (NaOH) were acquired from Sigma Aldrich. A variety of solvents—including acetone, acetonitrile, chloroform, dichloromethane, diethyl ether, ethanol, hexane, toluene, and *N,N*-dimethylformamide—as well as hydrochloric acid and acetic acid, were sourced from Merck India and utilized as received. Double distilled water was produced by distilling distilled water over alkaline potassium permanganate.

3. Methods :

3.1 Selection and Collection of Plants

The Indian herbal plant *Aervalanata* was collected from the campus of our institution. The dried and waste parts of the plant were meticulously separated. Subsequently, the collected specimens were washed with tap water and cut into smaller pieces. To prevent the degradation of phytoconstituents due to sunlight exposure, the plant materials were air-dried completely under shade at room temperature over seven days. Once adequately dried, the materials were processed into a powder using a pulverizer and were then sieved to a mesh size of 120. The resultant fine powder was homogenized and stored in an air-tight container for subsequent analysis.

3.2 Preparation of water extracts of *Aervalanata* plants

The preparation of plant extracts involves a constructive approach, starting with the careful mixing of 10 g of freshly dried plant powder with 200 ml of distilled water in separate batches. This mixture is then heated at 80°C for 2 hours with continuous stirring, facilitating optimal extraction. Following this process, the resulting residue is effectively filtered out using Whatman No. 42 filter paper. The extracts are stored at 4°C, providing a stable solution for the subsequent synthesis of chromium oxide nanoparticles.

3.3 Solubility test

Approximately 0.1 grams of the substance was placed in a clean 10 ml test tube, and 1 ml of a low-polarity solvent, such as benzene or petroleum ether, was added before shaking the mixture thoroughly. If the substance remained insoluble, the procedure was repeated using solvents with increasing polarity, including acetone, acetonitrile, toluene, ethanol, and others. The solubility of the substance was then assessed with high-polarity double-distilled water. Once solubility was confirmed, an additional 0.1 grams of the non-polar substance was introduced until the saturation limit was reached. This process allowed us to determine the saturation or solubility limit of our nanoparticles.

3.4 UV-visible Absorption Spectrum

The electronic spectra were recorded in the 200-900 nm range using a Deep Vision UV/VIS spectrophotometer and a cuvette with a 1 cm path length. The concentrations of the ligand and metal complexes were consistently maintained at 1.00×10^{-5} mol/L at a temperature of 310 K.

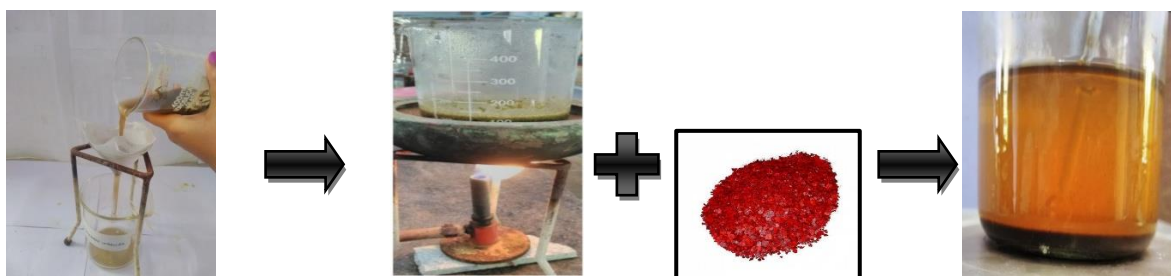
3.5 Antibacterial activity

The disc diffusion method was employed to assess the antibacterial capabilities of plant-derived metal nanoparticles (NPs) against several bacterial strains, including *Bacillus subtilis* (BS), *Bacillus cereus* (BC), *Staphylococcus albus* (SA), *Pseudomonas aeruginosa* (PA), *E. coli*, and *Klebsiella pneumoniae* (KP). Before conducting the experiments, the bacterial strains were incubated overnight in nutrient broth and maintained at 37 °C for 24 hours. To verify the antibacterial efficacy of the NPs, an overnight bacterial culture was spread onto prepared agar plates and allowed to dry for 5 minutes. Additionally, filter discs that contained varying concentrations of NPs (ranging from 1 to 10 µg/ml) were dried and placed on the surface of the agar. The plates were then placed in an incubator and monitored for zones of inhibition (ZOI). Streptomycin was utilized as the positive control, while DMSO served as the negative control.

4. Result and Discussion :

4.1. Green Synthesis of Chromium Oxide nanoparticle

A salt solution was prepared by dissolving 1 g of $[\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}]$ in deionized water, which was subsequently combined with 30 mL of green extract from *Aervalanata*. This mixture was stirred at a temperature of 80°C for 2 hours. To achieve and maintain a pH level of 14, a dilute solution of sodium hydroxide (0.01 mM) was added incrementally. Following this, the resultant mixture was concentrated and left in a dark environment for 48 hours. The resulting crystals were then dried in a hot air oven at 90°C for 2 hours. The dried crystals were subjected to calcination at 400°C in a muffle furnace for 3 hours, leading to the formation of a dark greenish-orange crystalline powder of Cr_2O_3 NP, achieved at a yield of 97%. The reaction process is illustrated in Scheme 1.



Scheme 1. Synthesis of Aervalanata extract-based Chromium Oxide nanoparticle

4.2 Saturation limit analysis

Approximately 0.1 grams of chromium(III) oxide nanoparticles (Cr_2O_3 NPs) were placed in a clean 10 ml test tube, followed by the addition of 1 ml of double-distilled water. The mixture was stirred until a clear solution was obtained, confirming the compound's solubility in the aqueous solvent. While maintaining stirring, an additional portion of 0.1 grams of Cr_2O_3 nanoparticles was introduced until the saturation limit was reached. This procedure established that the saturation limit of the nanoparticles in water is 0.7 grams per 1 ml.

4.3 UV-Visible spectroscopy

The synthesis of chromium oxide nanoparticles was validated using UV-VIS spectrophotometry. As illustrated in Figure 2, the UV-Vis absorption spectrum for the chromium oxide nanoparticle sample was obtained over a wavelength range of 200–800 nm. The spectrum exhibited a prominent absorbance band at 318 nm, along with a shoulder band at 351 nm, which are indicative of the characteristic optical transitions associated with chromium oxide nanoparticles.

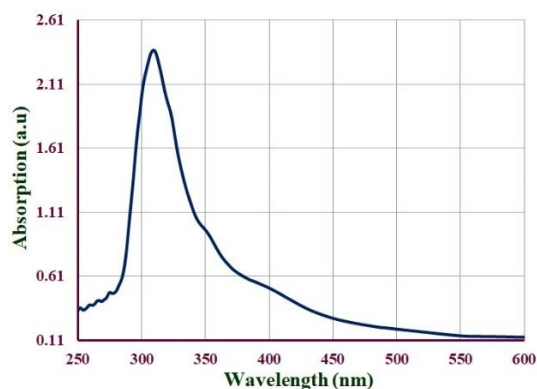


Figure 2. UV-visible spectrum of Chromium oxide nanoparticle

4.4. Surface morphological study

The investigation into the synthesized Cr_2O_3 nanoparticles derived from Aervalanata extracts has yielded impressive results, showcasing their remarkable characteristics. Through the use of Scanning Electron Microscopy (SEM), we conducted a thorough analysis of the surface morphology, and the findings were compelling. The SEM images demonstrate that the nanoparticles predominantly adopt spherical shapes, with an average diameter of 33 nanometers. This distinctly highlights the significant influence of Aervalanata extract in the synthesis process, producing nanoparticles with sizes ranging from 27 to 40 nanometers. Moreover, the outstanding dispersibility of these Cr_2O_3 nanoparticles is a crucial feature that enhances their utility across various applications. The presence of a distinct coating layer observed in the SEM images strongly indicates that the phytochemicals from Aervalanata serve as effective capping agents. This is a critical finding, as these phytochemicals play a vital role in stabilizing the nanoparticles during synthesis and preventing agglomeration. Overall, these compelling attributes position Cr_2O_3 nanoparticles as a highly promising material for advancing various technological innovations.

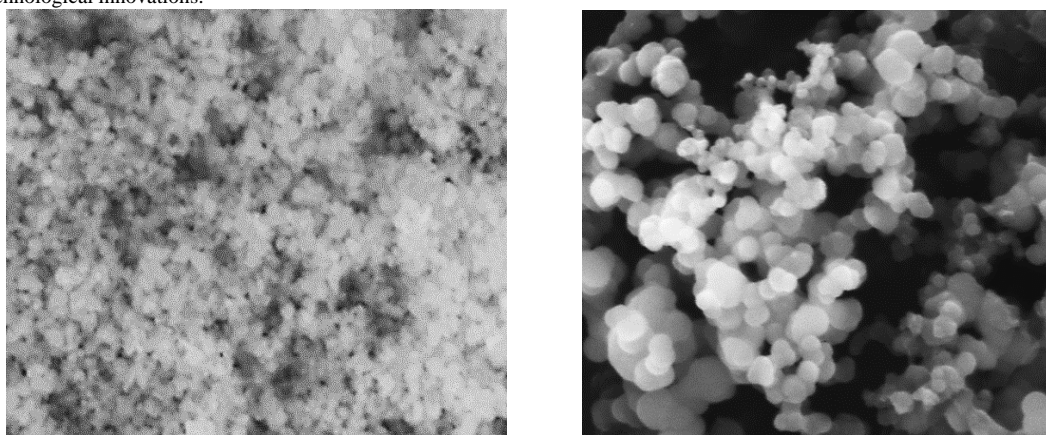


Figure 3. SEM image of Chromium oxide nanoparticle

4.5 Antibacterial activity

The antibacterial activity of Chromium oxide nanoparticles was evaluated against Gram-negative and Gram-positive bacteria at various concentrations of the samples. The study involved the determination of the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and assessment of the zone of inhibition. A specified volume of bacterial strains, including *Bacillus subtilis* (BS), *Bacillus cereus* (BC), *Staphylococcus albus* (SA), *Pseudomonas aeruginosa* (PA), *Escherichia coli* (E. coli), and *Klebsiella pneumoniae* (KP), was added to each sample in physiological serum to achieve a target concentration of 100,000 bacteria per mL. The samples were then incubated at 37 °C. Control groups, both positive and negative, were also established for comparative purposes. The disk diffusion method was employed to quantify the zone of inhibition, utilizing various sample concentrations placed at designated distances within the agar medium. Subsequently, the disks were incubated at 37 °C for 24 hours. The diameter of the zone of inhibition was measured accurately using a ruler. The results indicated that *Bacillus subtilis* (BS), *Pseudomonas aeruginosa* (PA), *Escherichia coli* (E. coli), and *Klebsiella pneumoniae* (KP) are viable candidates for exhibiting the antibacterial activity of Chromium oxide nanoparticles, as illustrated in Figure 4 and Table 1. The results demonstrated that a concentration of 9 $\mu\text{g/ml}$ of the green Cr_2O_3 NPs displayed significant efficacy against these bacterial strains, which can be attributed to the presence of heterocyclic compounds derived from the plant.

Table 1. Showing the results of zone inhibition of chromium oxide with Bacteria's

S. No.	Bacteria	Chromium oxide nanoparticles @ 9 $\mu\text{g/ml}$	Control (STREPTOMYCIN)
1.	BS	19	22
2.	BC	5	20
3.	PA	13	12
4.	SA	3	12
5.	KP	15	13
6.	E.coli	16	11

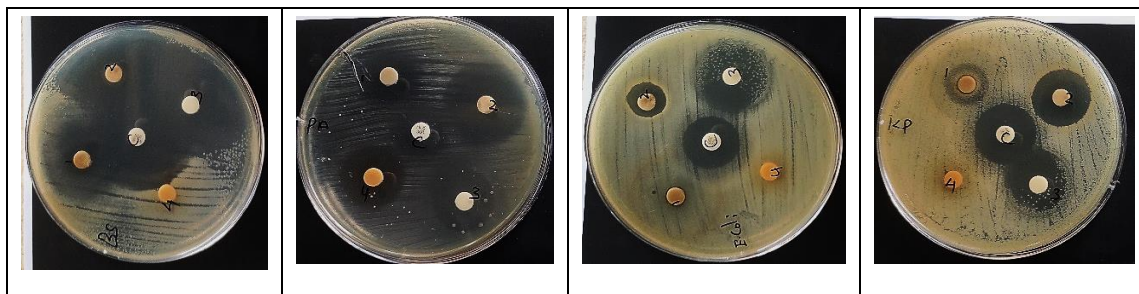


Figure 4. Antibacterial activity of chromium oxide nanoparticle

5. Summary and future scope :

Chromium oxide (Cr_2O_3) nanoparticles were synthesized using a green extract from *Aervalanata*, achieving a synthesis yield of 92%. The characterization of the nanoparticles was confirmed via UV-Vis spectroscopy, which displayed a distinct absorbance peak at 318 nm, indicative of chromium oxide. Scanning Electron Microscopy (SEM) imaging revealed that the nanoparticles predominantly exhibited spherical morphologies with an average diameter of approximately 34 nanometers. The size distribution of the Cr_2O_3 nanoparticles ranged from 27 to 40 nanometers, highlighting the effective influence of the *Aervalanata* extract on the synthesis process. In terms of dispersibility, the green-synthesized Cr_2O_3 nanoparticles demonstrated excellent characteristics, enhancing their potential application across various fields. Their antibacterial efficacy was assessed against six pathogenic bacterial strains, inclusive of both Gram-positive and Gram-negative species, in comparison to a standard antibiotic. Results indicated that all tested concentrations exhibited concentration-dependent antibacterial activity, with significant bacterial growth inhibition noted at 9 $\mu\text{g/mL}$. Notably, the green-synthesized Cr_2O_3 nanoparticles exhibited superior zones of inhibition (ZOIs) against both bacterial classes compared to the standard drug.

The study revealed effective antibacterial activity against several strains, including *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. This differential susceptibility observed among the bacterial strains may be attributed to variances in the chemical composition and structural characteristics of their cell walls. For instance, Gram-negative bacteria possess a cell wall comprised of lipopolysaccharides, lipoproteins, and phospholipids, while Gram-positive bacteria contain a more straightforward structure of peptidoglycan and teichoic acid, featuring larger pore sizes. The small dimensions of the nanoparticles facilitate their penetration through these cell walls, allowing for disruption and subsequent bacterial lysis. Further examination revealed that treated bacterial cells exhibited a red coloration, indicating a compromise in membrane permeability and integrity catalyzed by the synthesized Cr_2O_3 nanoparticles. Consequently, this interaction leads to the cell's ultimate demise, underscoring the potential of green-synthesized metal oxide nanoparticles in antibacterial applications.

6. Conclusions :

We successfully synthesized green plant-mediated Cr_2O_3 nanoparticles using a calcination method, which is simple, cost-effective, and environmentally friendly. The nanoparticles were characterized by UV-visible spectroscopy and SEM, revealing spherical shapes with an average diameter of 33 nanometers, influenced by *Aervalanata* extract, which produced sizes ranging from 27 to 40 nanometers. These nanoparticles have excellent dispersibility and antimicrobial efficacy against standard bacterial strains, with improved antibacterial activities due to their smaller size and increased surface area. The *Aervalanata* extract also shows strong antibacterial properties which enhances the biological activities of the nanoparticles. Our findings indicate that these green synthesized Cr_2O_3 nanoparticles have potential for various biomedical applications, including antifungal and larvicidal uses. This method is efficient and cost-effective for producing biocompatible nanoparticles with reduced toxicity and improved safety for human health. To ensure consistent therapeutic outcomes, standardization through pharmacokinetic studies should be conducted to maintain the appropriate dosage for effectiveness.

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