Enhanced Photocatalytic Degradation and Antimicrobial Activities of Biogenic Co_3O_4 Nanoparticles Mediated by Fenugreek: Sustainable Strategies

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1. Experimental Details

1.1. Materials

Cobalt nitrate hexahydrate ($Co(NO_3)_2.6H_2O$), sodium hydroxide (NaOH), and the organic solvent ethanol (EtOH) were purchased from Sigma-Aldrich, Germany, and were of analytical grade. Deionized water was used throughout the experiment. Trigonella foenum-graecum (TFG) seeds were collected from the local market of Fatehgarh Sahib, Latitude 30.6435° N, Longitude 76.3970° E of Punjab, India.

1.2. Preparation of TFG seed aqueous extract

The first crucial step in TFG seed extract preparation involves cleaning the seeds with tap water, aiming to eliminate any potential sources of contamination such as dirt and debris. The choice of tap water is grounded in its general acceptability and safety for drinking, ensuring a pristine starting point for the subsequent extraction process. This careful cleaning process is imperative to safeguard the purity of the final extract and establish a clean baseline. Following the cleaning phase, the seeds undergo sun-drying, a step justified by its role in dehydration, preservation, and prevention of microbial growth. Sunlight exposure activates beneficial compounds within the seeds, setting the stage for the development of a concentrated and

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biologically active extract. This pivotal step contributes to the overall potency of the extract and establishes a foundation for subsequent processing. Next, the dried seeds are finely ground into a powder, a critical step justified by its capacity to increase the surface area. This enhancement facilitates improved contact with the solvent, typically ethanol, during the extraction process. The grinding process enhances the efficiency of compound extraction, ensuring a comprehensive and potent final extract with optimal bioactive constituents. Subsequently, the TFG seed powder (20 g) undergoes incubation in ethanol (200 mL) at room temperature for 72 hours. Ethanol, chosen for its solvent properties, selectively extracts a broad range of compounds from the seeds. The extraction is conducted at room temperature to optimize the process without compromising heat-sensitive components. The 72-hour incubation period allows for thorough and complete extraction of the desired compounds, ensuring a robust and comprehensive extract. Following incubation, the mixture undergoes filtration using filter paper (Whatman). This method effectively separates the extracted liquid (filtrate) from the solid plant material (residue). Whatman filter paper is chosen for its ability to retain coarse plant debris, guaranteeing a clean and clarified liquid extract for subsequent analysis and experimentation. The final step involves storing the filtrate at a low temperature of 4 °C. This strategic decision is justified by its role in minimizing degradation, thereby preserving the activity and effectiveness of the extracted compounds. The low temperature ensures the stability of the extract over extended periods, facilitating comprehensive experimentation and analysis without compromising the quality of the extract.

1.3. Green synthesis of Co_3O_4 NPs

The biosynthesis of Co_3O_4 nanoparticles (NPs) follows a modified procedure based on a referenced study [1]. Initially, 2.91 g of cobalt nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$) was accurately weighed using an analytical balance and dissolved in 20 mL of distilled water. The solution was stirred at 500 rpm for 30 minutes using a magnetic stirrer with a Teflon-coated stirring bar to ensure complete dissolution of the cobalt nitrate, leaving no visible undissolved particles.

A total of 50 mL of prepared filtrate was measured using a graduated cylinder and added to the cobalt nitrate solution. The mixture was heated to 60-70 °C using a hot plate, with continuous pH monitoring to maintain a pH of 8, adjusted by the addition of 1 M NaOH solution

as necessary. The temperature was controlled within the specified range, and stirring was maintained at 500 rpm for an additional 30 minutes to promote effective interaction between the cobalt ions and the biomolecules present in the plant extract. During this period, a color change from pink to dark brown was observed, typically within the first 15-20 minutes, indicating the reduction of cobalt ions and the formation of Co_3O_4 NPs.

After allowing the reaction mixture to cool to room temperature (28 °C), it was left undisturbed for 24 hours to ensure complete precipitation of the nanoparticles. The dark brown precipitate was then collected by centrifugation at 5000 rpm for 10 minutes. The precipitate was washed three times with 20 mL of distilled water, followed by washing with 20 mL of ethanol, to remove any unreacted plant extract and residual ions.

The washed precipitate was dried in a hot air oven at 100 °C for 4 hours, ensuring uniform drying by spreading the sample thinly on a petri dish. Finally, the dried sample was subjected to calcination at 180 °C for 2 hours in a muffle furnace to enhance crystallinity. The temperature was gradually increased at a rate of 5 °C/min to prevent thermal shock, thereby completing the biosynthesis process.

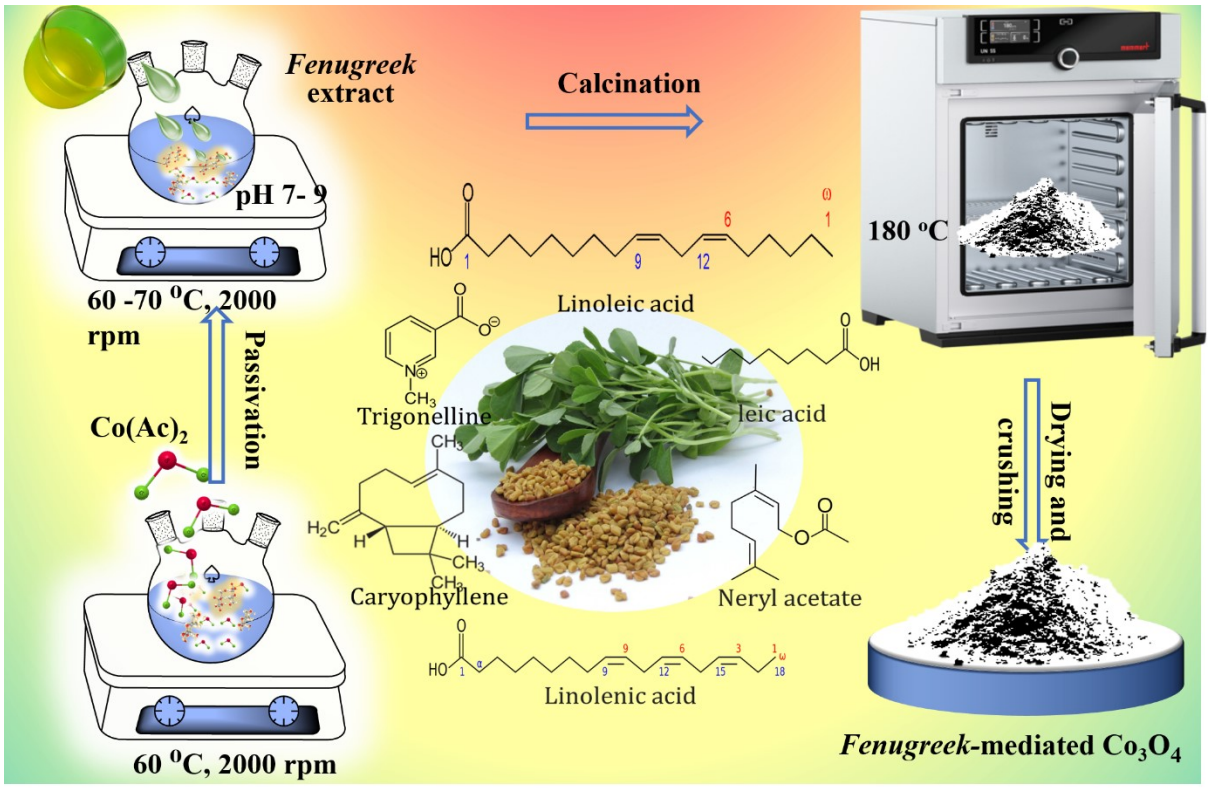

Figure 1. Synthesis of *Trigonella foenum-graecum*-mediated Co_3O_4 nanoparticles.

2. Experimental details for photocatalytic activity

2.1. Materials

Congo Red (CR), the sodium salt of benzidinediazo-bis-1-naphthylamine-4-sulfonic acid ($C_{32}H_{22}N_6O_6S_2Na_2$, MW: 696.7 g/mol), served as a valuable model pollutant in this study. This diazo dye, procured from Merck, possesses a unique aromatic structure, sulfonic acid groups, and azo-bond linkage, making it particularly suitable for investigating its removal using the photocatalysis method. The intricate molecular structure of Congo Red showcased in Figure 2, plays a crucial role in its properties and behavior, facilitating adsorption and degradation mechanisms essential for pollutant removal. Understanding the interaction between this dye and the proposed treatment method requires a deep dive into its chemical makeup.

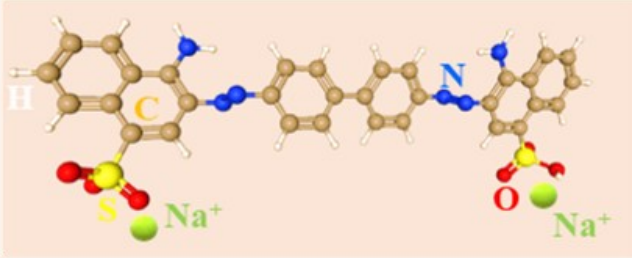

Figure 2. Structure of CR dye.

2.2. Photocatalytic activity

The photocatalytic activity of TFG-mediated Co_3O_4 nanoparticles (NPs) was assessed for the degradation of Congo Red (CR) dye under ultraviolet (UV) light irradiation. This evaluation aimed to elucidate the potential of these NPs as an effective and sustainable treatment option for dye-contaminated wastewater. The UV light source utilized in the study was an 8W UV lamp emitting UVB light within the wavelength range of 280-315 nm. This specific UVB range is relevant for Co_3O_4 photocatalysis, as it aligns with the bandgap excitation of the nanoparticles. Two different catalyst concentrations (100 and 200 mg/L) were systematically employed in the experiment, allowing for a comprehensive exploration of dose-dependent effects on photocatalytic activity. The experimental procedure involved an initial dark adsorption step, where the NPs were dispersed in the CR dye solution and subjected to magnetic stirring in complete darkness for 1 hour. A 4 mL sample was then extracted to assess adsorption equilibrium before UV light exposure. The subsequent activation of the UV lamp initiated the photocatalytic process, with periodic sample collection during light exposure. To ensure accurate measurement of CR concentration, samples underwent centrifugation to remove the catalyst before UV-Vis analysis. The UV-Vis spectrum displayed the maximum absorbance peak of CR dye at 497 nm, allowing for the determination of concentration changes.

The degradation efficiency of the photocatalytic process was quantified by calculating the percentage removal of CR dye

$$\% \text{ Removal of CR dye} = ((C_0-C_t)/C_0) * 100 \quad (1)$$

Here, C_0 represents the initial dye concentration, and C_t represents the dye concentration at a specific time point (t).

The study also considered the initial concentration of CR, ensuring relevant context for the experiments. Furthermore, the timeframe and frequency of sample collection during light exposure were addressed to provide a comprehensive understanding of the experimental setup. Specific features of the UV-Vis spectrophotometer were also considered in the experimental design.

2.3. Antimicrobial investigation

The antimicrobial efficacy of TFG-mediated Co_3O_4 NPs was investigated using the agar well diffusion method, a standardized technique providing precise insights into their effectiveness against bacterial pathogens. This experimental setup was conducted on petri dishes, where nutrient-rich broth facilitated bacterial growth, and solidified agar served as the substrate. *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*), representative Gram-positive and Gram-negative pathogens, were employed to assess the broad-spectrum antimicrobial capabilities of the TFG-mediated Co_3O_4 NPs, common hospital-acquired strains with distinct cell wall defenses. Employing a sterile cork borer, wells were strategically created on the agar, acting as confined arenas for the confrontation between the NPs, at varied concentrations (e.g., 10 µL, 25 µL, and 40 µL), and the bacterial adversaries. This design facilitated the determination of the minimum inhibitory concentration (MIC), representing the lowest NP dose effectively halting bacterial growth. Following a 24-hour incubation period at 37 °C, the resultant "inhibition zones" around each well was precisely measured, providing a quantitative measure of the NPs' effectiveness. Larger zones indicated more pronounced inhibitory effects. Beyond a mere experimental victory, this agar well diffusion assay offers valuable insights into the potential applications of TFG-mediated Co_3O_4 NPs in disinfectants, coatings, and medical treatments, presenting a paradigm where nanotechnology emerges as a formidable strategy against resilient bacterial threats.

3. Characterization of Co_3O_4 NPs

Following the biosynthetic process, the Co_3O_4 NPs were precisely characterized to understand their structural, morphological, and optical properties. This in-depth analysis employed a battery of sophisticated instruments, unveiling the inner workings of these microscopic marvels. X-ray diffractometer (model no.: DY 3190, PANalytical X'PERT-PRO, Japan) using Copper-Kα radiation with a wavelength of 1.54 nm was utilized for the XRD

spectrum. It was recorded within the 2θ range of 30° to 70° and instrument was operated at accelerating voltage of 45kV with small current of 40 mA. This analysis provided crucial information about the crystal structure of the nanoparticles, revealing their atomic arrangement and confirming the formation of the desired Co_3O_4 phase. Fourier transform infrared (FTIR) spectrometer (model no.: 200695, Alpha- Bruker, Germany), was employed in this investigation and it scanned the vibrational fingerprints of the NPs in the 500 to 4000 cm^{-1} range. This technique served as a powerful tool to identify the functional groups present on the surface of the Co_3O_4 NPs. To investigate the world of the infinitely small, a field emission scanning electron microscope (FESEM) of SUPRA 55VP, Carl Zeiss, Germany, was employed and it offered a high-resolution glimpse into the surface morphology of the Co_3O_4 NPs. For FESEM analysis, a suspension of Co_3O_4 powder (10 mg) in ethanol was prepared using ultra bath sonicator. To complete the characterization, energy dispersive X-ray spectroscopy (EDX) of enigma was used. This technique, integrated within the FESEM, provided elemental analysis of the Co_3O_4 NPs. By studying the characteristic X-ray emissions triggered by the electron beam, the presence of cobalt and oxygen as expected, as well as the detection of any trace elements incorporated into the NPs during the biosynthesis process. Additionally, high-resolution transmission electron microscope (HRTEM) of JEM-2100 (JEOL, Japan) provided high-resolution images for precise quantification of particle dimensions, lattice parameters, and interplanar spacing. For HRTEM analysis, a drop of suspension of Co_3O_4 (10 mg of Co_3O_4 and ethanol) was placed on a carbon grid. Selected area diffraction (SAED) pattern was also obtained from HRTEM using a selected area aperture which is positioned below the sample holder in the column of HRTEM. The light-absorbing properties of the Co_3O_4 NPs were investigated using a UV-visible spectrometer (UV-2600, Shimadzu, Japan). This instrument recorded the absorption spectrum of the NPs in the wavelength range of 200 to 800 nm. The specific wavelengths at which the Co_3O_4 NPs absorbed light provided valuable insights into their electronic band structure and potential applications in fields like photocatalysis and optoelectronics. X-ray photoelectron spectroscopy (XPS) analysis was performed on a PHI 5000 VersaProbe II instrument (ULVAC-PHI, Inc.) to investigate the chemical state of the Co_3O_4 NPs. The investigation of control CR dye and its fragments formed during the photodegradation under UV irradiation was carried out using a gas chromatograph (GC: TRACE 1310 GC, Thermo Fisher Scientific, Inc., USA) attached with a mass spectrometer (MS: TSQ 8000 Evo Triple Quadrupole Mass Spectrometer, Thermo Fisher Scientific, Inc.,

USA). The temperature of the sample injector was set to 280 °C, and the splitless mode injection volume was 1 microliter while the splitless duration was set to 1 minute.

Reference

1. Kumar, S., et al., *Potential of Piper betle@Co3O4 nanoparticles as high-performance photocatalysts for the removal of industrial dyes.* Journal of Cleaner Production, 2022. **361**: p. 132242.