RESEARCH ARTICLE

Nickel Oxide Nanoparticles via Green Synthesis: Insights into Antimicrobial, Antifungal and Dye Degradation Potentials

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Abstract

Alternanthera sessilis aqueous extract was utilized to synthesize nickel oxide nanoparticles through a biological route that is simple and cost-effective. The leaves of *A. sessilis*, consumed as a vegetable, are renowned for their numerous health benefits. They are believed to promote hair growth, aid digestion, reduce inflammation, relieve joint pain, improve eyesight, and help treat conditions like leucorrhoea, diarrhea, skin diseases, fever, dyspepsia, and night blindness. This extract aids in preventing agglomeration and acts as a capping agent. The nickel oxide nanoparticles (NiONPs) were characterized using photoluminescence excitation and emission spectra, recorded with a Shimadzu RF-5301 PC spectrofluorometer. Additionally, UV-visible spectroscopy was employed to obtain absorbance spectra, confirming the formation of NiONPs. X-ray techniques were also used to analyze other properties of the synthesized nanoparticles. Additionally, the synthesized nanoparticles were evaluated for their antimicrobial activity against *Bacillus subtilis*, antifungal activity against *Candida albicans*, and dye-reduction efficiency against Rhodamine B and Methylene Blue. The results indicate their potential as effective agents for antimicrobial, antifungal, and dye-removal applications.

Highlights

- Nickel oxide nanoparticles (NiO NPs) were synthesized using an eco-friendly approach with *Alternanthera sessilis* leaf extract as a reducing and stabilizing agent.
- Advanced analytical techniques, including scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX), were employed to analyze the morphology, elemental composition, and structural properties of the nanoparticles.
- NiO NPs demonstrated significant antimicrobial and antifungal activity against *Bacillus subtilis* and *Candida albicans,* respectively, showcasing their potential for medical and industrial applications.
- The catalytic performance of NiO nanoparticles (NPs) was evaluated for the degradation of hazardous dyes, such as rhodamine-B and methylene blue, highlighting their potential for environmental remediation.
- The study highlights the importance of green chemistry principles in nanoparticle synthesis, thereby reducing reliance on toxic chemicals.
- The findings highlight the dual utility of NiO NPs in combating microbial contamination and addressing industrial dye pollution.

Keywords: Green synthesis, *Alternanthera sessilis* Nickel oxide nanoparticles, Photoluminescence (PL), antibacterial activity, antifungal activity, dye reduction.

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Introduction

Nanotechnology involves the manipulation of matter at the atomic, molecular, and supramolecular levels, typically within the size range of 1 to 100 nanometers (Gupta & Sahay, 2018). In recent years, there has been a growing interest in nanoscience and nanotechnology, resulting in the development of various nanomaterials (Sharma *et al*., 2022). Due to their extremely small size and high surface area-to-volume ratio, these materials exhibit unique properties that enable a wide range of applications (Thakur & Thakur, 2022). Nanomaterials are now used across diverse fields, including materials science, biotechnology, microbiology, environmental remediation, optics, electronics, mechanics, and medicine (Gopi & Amalraj, 2016).

Plant-derived materials have been used for a long time to improve our quality of life (Kumar & Seth, 2021). These biostimulants, developed through micro- and nanotechnology, can be applied effectively without posing risks of pollution or environmental harm (Selvanathan *et al*., 2021). It is crucial to establish guidelines for their development, application, and effective environmental monitoring. Plants act as synergistic medicines; their components can enhance the effectiveness of medicinal treatments (Some *et al*., 2021). For instance, medicinal plants such as ginger, turmeric, green tea, and walnuts have been shown to help control diseases and reduce the harmful effects of chemical treatments (Zhang *et al*., 2021).

The synthesis of nanoparticles (NPs) using physical and chemical methods can be both time-consuming and expensive. Additionally, the chemicals involved in these synthesis processes can be harmful to the environment and pose biological risks. As a result, green synthesis has gained increasing attention as an

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alternative method for producing NPs (Gour & Jain, 2019). Plant extracts can act as both reducing and stabilizing agents (Kavitha *et al*., 2024) *Lindl. Leaf Extract and Evaluation of Biological Activity-An Endangered Orchid, 2024*) in this process. Plant extracts contain various phytochemicals, such as terpenoids, flavonoids, alkaloids, phenolics, and saponins, which serve as reducing and stabilizing agents(Donga & Chanda, 2021). In recent years, various parts of plants have been explored for the synthesis of metal nanoparticles (NPs) (Pote *et al*., 2024). The green synthesis of nanomaterials is not only straightforward and less expensive compared to traditional physical and chemical methods but also more environmentally friendly (Louafi *et al*., 2022).

Industrial wastewater from various sectors contains many contaminants, including insecticides, peroxides, heavy metals, antibiotics, and harmful chemical dyes. These dyes are significant pollutants found in wastewater discharged by factories involved in the production of textiles, plastics, paper, food, tanneries, and pharmaceuticals, among other industries. Alongside these, other harmful chemical substances are also present in the wastewater. (Bansal *et al*., 2020). Some of the primary dyes used in industries for staining include azo, basic, acidic, and cationic dyes (Samuchiwal *et al*., 2021). Synthetic dyes are highly toxic, mutagenic, and carcinogenic. Industries often discharge wastewater containing these dyes into nearby bodies of water, such as lakes, rivers, and drains. Furthermore, some companies use this wastewater for irrigation, which can deteriorate soil and crop quality. This practice also reduces sunlight penetration into water bodies, negatively affecting aquatic organisms (Ikram *et al*., 2020) & (Stone *et al*., 2020). It is essential to treat effluent-containing dyes properly before discharging it into nearby water sources. The chemical structure of dye molecules confers significant stability, making it challenging to decompose them into harmless components.

The green synthesis of metallic nanoparticles possesses multiple physical and chemical properties (Garg *et al*., 2020). The use of these metallic nanoparticles for degrading toxic dyes has recently gained significant attention due to their catalytic activity. Numerous studies have demonstrated the catalytic effectiveness of nanoparticles synthesized through green methods against various toxic dyes, including Indigo carmine, tymol blue, auramine O, rhodamine B, congo red, methyl orange, methylene blue, etc, (Chandhru *et al*., 2020). In the present study, rhodamine-B (Rh-B) and methylene blue (MB) were selected because they are common synthetic dyes widely used in the textile, paper, and plastic industries. Additionally, their toxic, persistent, and non-biodegradable nature makes them ideal representatives of hazardous industrial pollutants in wastewater (Khairnar & Shrivastava, 2019). Degradation rates of 90 to 100% have been observed for dyes utilizing nickel oxide nanoparticles. Additionally, the development of nanoparticle-based tint deprivation is rapid and free of slightly dangerous chemicals associated with chemical and biological wastewater treatment processes. Metal nanoparticles possess exceptional chemical, electronic, and physical properties that make them excellent catalysts for the degradation of organic dyes through reductive processes (Edison *et al*., 2016). They provide an effective alternative to outdated methods for removing tint impurities. Currently, green-synthesized nickel oxide nanoparticles are being extensively utilized to reduce tint concentrations in aqueous solutions.

Alternanthera sessilis is a prostrate herb that can be either annual or perennial. It features several spreading branches, short, petioled, simple leaves, and small white flowers (Nikam & Namdas, 2022). This plant is found throughout the hotter regions of India, growing at altitudes of up to 1,200 meters (Pathak *et al*., 2020). *A. sessilis*, commonly known as dwarf copperleaf, purple mint, sissoo spinach, or sessile joyweed, is a versatile aquatic and semi-aquatic plant belonging to the family Amaranthaceae. The plant is recognized for its medicinal properties in traditional medicine, being utilized to treat various ailments, including fevers, wounds, and digestive issues (Chandrashekhar, 2020). Various studies have shown that the *A. sessilis* extract exhibits several pharmacological activities such as antibacterial, antiulcer, anthelminthic, antifertility, antibiofilm, antioxidant, and many more (Kota *et al*., 2017). These activities are exhibited by the *A. sessilis* extract because of the presence of flavonoids, anthraquinones, glucosides, alkaloids, etc. (Pathak *et al*., 2020). Various studies have consistently demonstrated that plants are generally rich in protein (Shreshtha *et al*., 2017).

The present work focuses on the synthesis of Nickel Oxide nanoparticles (NiONPs) using a green synthesis method (Shivay & Prasad, 2019). *A. sessilis* leaf extract was employed as a dropping and alleviating agent. Nickel Oxide nanoparticles are important transition nanoparticles with cubic lattice symmetry. NiO nanoparticles exhibit a diverse range of characteristics, including large specific capacitance, high catalytic activity, and notable cytotoxic, antibacterial, and antifungal properties(Nimisha *et al*.,2022).As a result, they are utilized in various applications, such as supercapacitors, gas sensors, dye reduction (Jeba & Amaladhas, 2018), electrochemical devices, and solar cells. Additionally, NiO nanoparticles have found widespread use in biomedicine due to their significant biological (Shanan & Shanshool, 2023) and therapeutic properties, which include a unique surface area and the ability to release and adsorb metal ions (Kganyago *et al*., 2018).

We synthesized NiO nanoparticles using the plant extract of *A. sessilis* to investigate their antimicrobial activity against *Bacillus subtilis*, antifungal activity against *Candida albicans* (Das & Kamble, 2024), and dye reduction activity on Rhodamine-B and Methylene Blue(Ahmad *et al*., 2023).

Materials and Methods

Collection of plant samples and extraction

Fresh plant samples of *A. sessilis* were collected from the Hudkeshwar area in Nagpur District, Maharashtra, between August and October. The plant was authenticated by a taxonomist from the Department of Botany at Rashtrasant Tukadoji Maharaj Nagpur University, and a herbarium specimen was submitted to the department **(voucher specimen no. 10922)**. The leaves were washed, dried, and then ground into a fine powder, which was sieved to collect the fine particles for further extraction. For the extraction process, 1 g of *A. sessilis* leaf powder was dissolved in 100 ml of distilled water in a 250 mL beaker. The mixture was stirred at 85°C for 45 to 60 minutes. Afterward, it was cooled to room temperature and filtered using

Whatman filter paper. The resulting extract was collected for further processing.

Synthesis of Nickel Oxide Nanoparticles

To synthesize nickel oxide nanoparticles (NiONPs) using *A. sessilis* leaf extract, 10 mL of the extract was added to 100 mL of a 0.5M Nickel Sulfate solution (NiSO4.6H2O). The solution was continuously stirred at 90°C for 3 hours. A color change indicated the successful synthesis of the Nickel Oxide nanoparticles (Fig 1). The NiONPs were then washed three times with water and centrifuged at 5000 rpm for 15 minutes. The resulting precipitate was dried at 75°C for 30 minutes. Finally, the precipitate was transferred to a crucible and calcined in a muffle furnace at 500°C for 3 hours.

Characterization technique

In this study, the X-ray diffraction pattern of the synthesized material was examined using a Rigaku MiniFlex X-ray diffractometer. The scanning range was set from 10 to 90° with a scanning rate of 0.02° per second. Additionally, the size of the particles and their morphological behavior were examined using images captured by a JSM-6360LV scanning electron microscope (SEM; JEOL, USA). The Fourier Transform Infrared (FT-IR) measurement of the synthesized nanoparticles was conducted using a Bruker Alpha FT-IR spectrometer. The photoluminescence (PL) properties were analyzed with a Shimadzu RF5301PC spectrofluorophotometer equipped with a 150-watt xenon flash lamp. A 10 nm spectral slit width was used to record the emission and excitation spectra of photoluminescence (PL), and the excitation source was a xenon lamp. Furthermore, the synthesized NPs were also evaluated for their antimicrobial and antifungal properties against *B. subtilis* and *C. albicans*, respectively, using a disc diffusion method and dye reduction activity with rhodamine-B and methylene blue.

Antibacterial and Antifungal Activity

The antimicrobial efficacy was assessed using the zone inhibition method, commonly known as the Kirby-Bauer method. Mueller-Hinton agar (MHA) plates were inoculated by spreading 100 µL of bacterial culture (*B. subtilis*) and fungal culture (*C. albicans*). The inoculum was prepared to achieve a 0.5 McFarland standard, which corresponds to about 1.5×10^8 CFU/mL of cell density. Discs containing 10 µL of dissimilar media (ranging from 10 µL–100 mg/mL) were placed on the agar plates. One disc on each plate contained solvent alone and served as the vehicle control. Additionally, discs containing 10 µg of ciprofloxacin and amphotericin B were employed as positive controls for antibacterial and antifungal activity, respectively. The plates with *B. subtilis* and *C. albicans* were incubated at 37°C for 24 hours. After cultivation, the strength of the surrounding districts was measured and recorded.

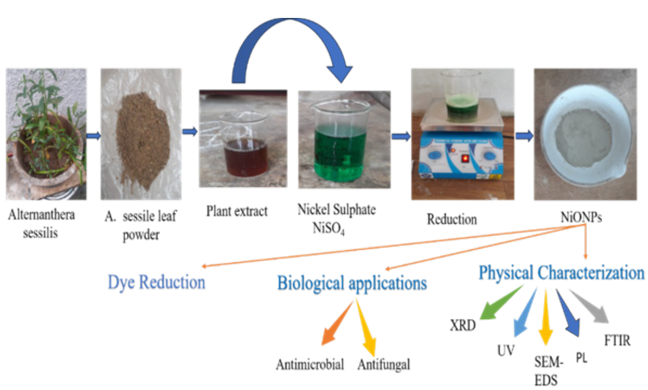

Fig. 1: Synthesis of nickel oxide nanoparticles from *A. sessilis* showing physical characterization and their biological applications

Catalytic reduction of dyes

To evaluate the effectiveness of nickel oxide nanoparticles (NiONPs), the ability of NiONPs to degrade rhodamine-B and methylene blue dyes in an aqueous solution was tested with sodium borohydride ($NaBH_4$) as a reducing agent. The final concentrations for all experimental studies, for both dye types, were 10 ppm. A 50 mM aqueous solution of $NaBH_4$ and an aqueous dye solution at 10 ppm were mixed in a 5 mL quartz cuvette and 50 µL of a solution containing NiONPs at an attentiveness of 0.05 mg/mL.

$$\text{Dye Degradation (\%)} = \frac{Ao - At}{Ao} \times 100$$

Where Ao is the initial absorbance and At is the absorbance at a time t'.

Results and Discussions

UV-vis Analysis

The UV-vis absorption bands of *Alternanthera sessile* NiONPs were recorded at different wavelengths from 200 to 800 nm. The broader peak was observed around 340 nm, Fig. 2. The broadened surface plasmon resonance peak of NiONPs indicates the presence of polydisperse nanosized particles.

Fourier transform infrared (FTIR) analysis

The FTIR ranges of *A. sessilis* nickel oxide nanoparticles are presented in Fig. 3. The presence of different functional groups is determined by FTIR analysis. In the present FTIR results, different peaks were noted at 3434, 3218, 1654, 1137, 982, 679, 593, 500 cm^{-1}. The peaks at 3434 to 3218 cm^{-1} are for the OH

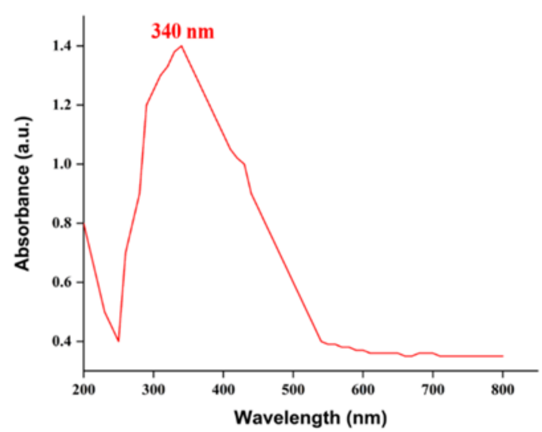

Fig. 2: UV-visible spectra of NiONPs

group, multiple peaks at 1654 cm^{-1} indicate C≡N bond, a band at 982 cm^{-1} shows a C-H stretching vibration, a band at 1137 cm^{-1} indicates C-O stretch the peak at 679 cm^{-1} signifies metal-oxygen stretching vibrations, confirming the presence of a Ni-O bond.

X-ray diffraction analysis

In Fig. 4 the crystallinity and structural details of *A. sessile* nickel oxide nanoparticles are obtainable. The X-ray diffraction pattern confirms the formation of NiO nanoparticles, with a strong reflection peak at 2θ = 20.56, 24.96, 35.08, 38.64, 45.4, 51.28, 66.76 corresponding to indices (101), (110), (111), (200), (202), (211), (220). The diffraction pattern for NiO NPs is consistent with JCPDS card number 1313-991. The obtained pattern reflected a mostly crystalline phase.

SEM and EDX analysis

The appearance of the biosynthesized surface of nickel oxide nanoparticles was examined with scanning electron microscopy at an amplification of 2 µm. As illustrated in Fig. 5(a) and (b), the nanoparticles show significant agglomeration, resulting in large and irregularly shaped particles forming uneven clusters. This agglomeration can be attributed to the increased surface energy and the amplified surface area-to-volume proportion.

The energy-dispersive X-ray (EDX) spectrum in Fig. 5(c) reveals distinct peaks at 0.9 keV and 7.5 keV, which are associated with nickel (Ni), while a peak at 2.5 keV indicates the presence of oxygen (O) and sulfur (S). These characteristic peaks confirm the successful synthesis of nickel oxide (NiO) nanoparticles.

Photoluminescence properties

The photoluminescence emission and excitation bands of the synthesized *A. sessilis* NiONPs were recorded. The PL excitation spectra were revealed at 277 nm. Under 277 nm excitation Fig. 6(a), the PL emission spectra were captured in the array of 362 nm Fig. 6(b). The recorded emission bands showed a broad emission peak in the green region.

Antibacterial and Antifungal Activity

The antimicrobial and antifungal activity of the *A. sessilis* leaf extract Nickel oxide nanoparticles was estimated in contrast to *B. subtilis* and *C. albicans* with the subsequent inhibition zone measurements (mm) presented in Fig. 7(a) and (b). Examination of the data revealed that the green synthesized instead of photogenic nickel oxide nanoparticles exhibited a maximum zone of inhibition of 25.67 mm for *B. subtilis* and 22 mm for *C. albicans*. In general, the antimicrobial potential was more pronounced against *B. subtilis* than *C. albicans*.

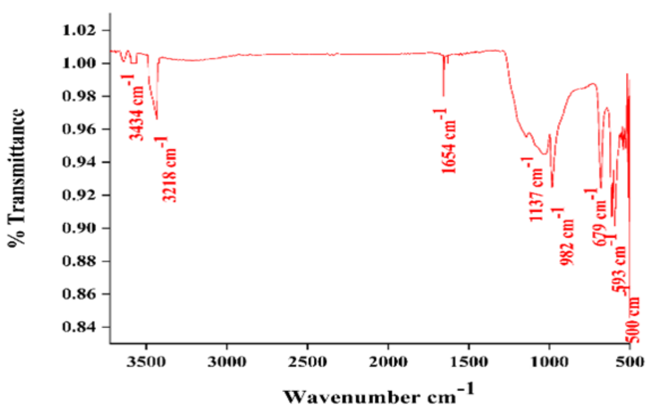

Fig. 3: FTIR spectra of the NiO nanoparticle

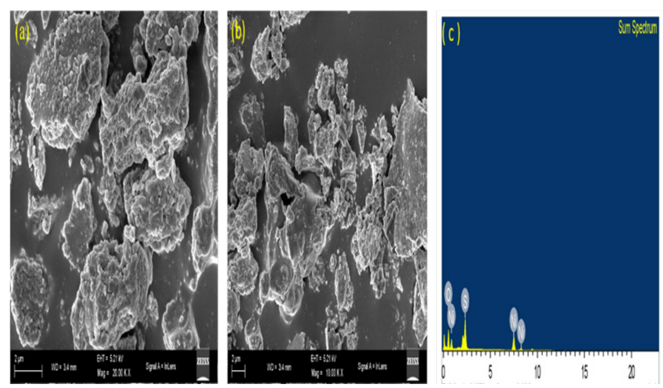

Fig. 5: (a) and (b) SEM image of *A. sessile* NiO NPs (c) EDX of A. sessilis NiO NPs

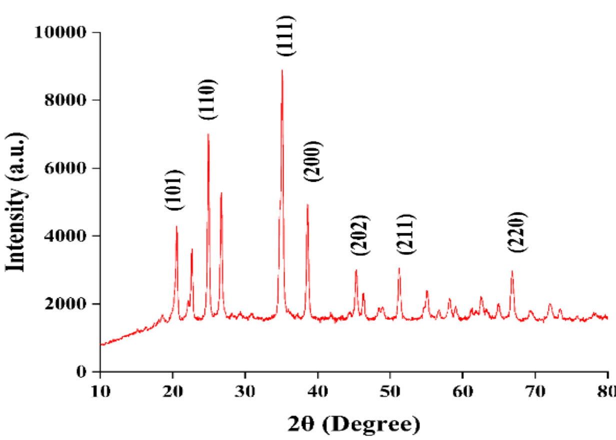

Fig. 4: XRD pattern of synthesized NiONPs

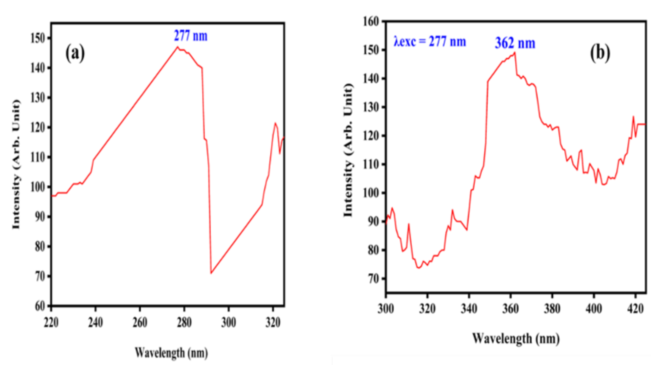

Fig. 6: (a) PL excitation under 277 nm excitation wavelength (b) PL emission spectra under 277 nm at 362 nm

Catalytic reduction of dyes

In this research work, we presented the activity of NiONPs in the deprivation of rhodamine-B and methylene blue dyes. The experiments were conducted in both the presence and absence of NiONPs to evaluate their potential as nanocatalysts with $NaBH_4$. In the dye reduction, $NaBH_4$ dissociates into the aqueous phase to form Na^+ and BH_4^- ions. Importantly, this dissociation occurs without a catalyst. The BH_4^- ions act as an electron source, which is crucial for the degradation of the dyes.

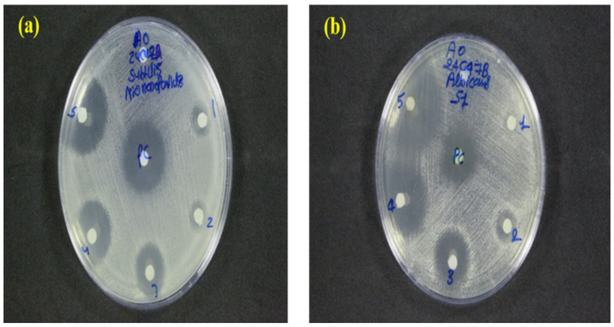

Fig. 7: Antimicrobial activity of NiO NPs against bacteria (a) *B. subtilis* and fungus (b) *C. albicans*

Rhodamine-B and methylene blue dyes are considered hazardous pollutants that often contaminate groundwater, particularly in areas near textile industries. Adding $NaBH_4$ to the dye solution does not substantially influence the degradation of the dye, Fig. 8(a) and Fig. 9(a). This is because the reduction of the dyes requires a more compatible redox potential, and $NaBH_4$ alone, with its distinct redox characteristics, is insufficient to facilitate the reduction process effectively (Velidandi *et al*., 2021).

The consequences demonstrated an important decrease in the dye concentrations when $NaBH_4$ was combined with NiO nanoparticles (NiONPs) as a nanocatalyst. This synergistic interaction between the reducing agent ($NaBH_4$) and the catalytic activity of NiONPs facilitated an efficient degradation of the dyes. Specifically, the decolorization of both rhodamine-B (Rh-B) and methylene blue was achieved within a remarkably short time of 270 seconds Fig. 8(b) and 9(b). The enhanced reduction rate can be attributed to the catalytic properties of NiONPs, which accelerate electron transfer from the BH_4^- ions generated by $NaBH_4$ to the dye molecules, breaking down their complex structures and leading to rapid decolorization. This highlights the effectiveness of NiONPs as a nanocatalyst in dye degradation processes, according to Wang *et al*. (2019).

The catalytic activity observed in the process can be attributed

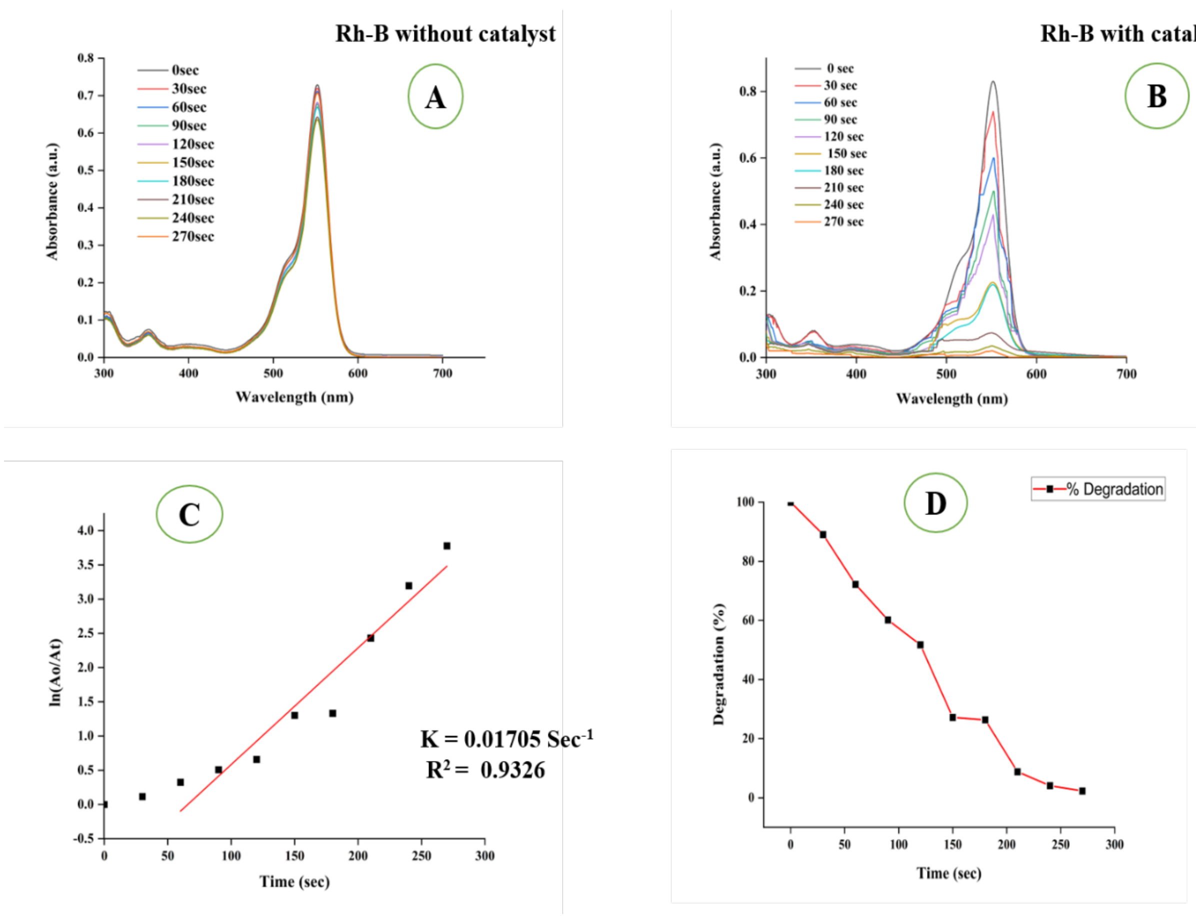

Fig. 8: Ultraviolet–visible spectra showing degradation of (A) Rhodamine-B dye without catalyst, (B) Rhodamine-B dye with catalyst, (C) first-order kinetics of catalytic reduction, (D) percentage of degradation with NiONPs.

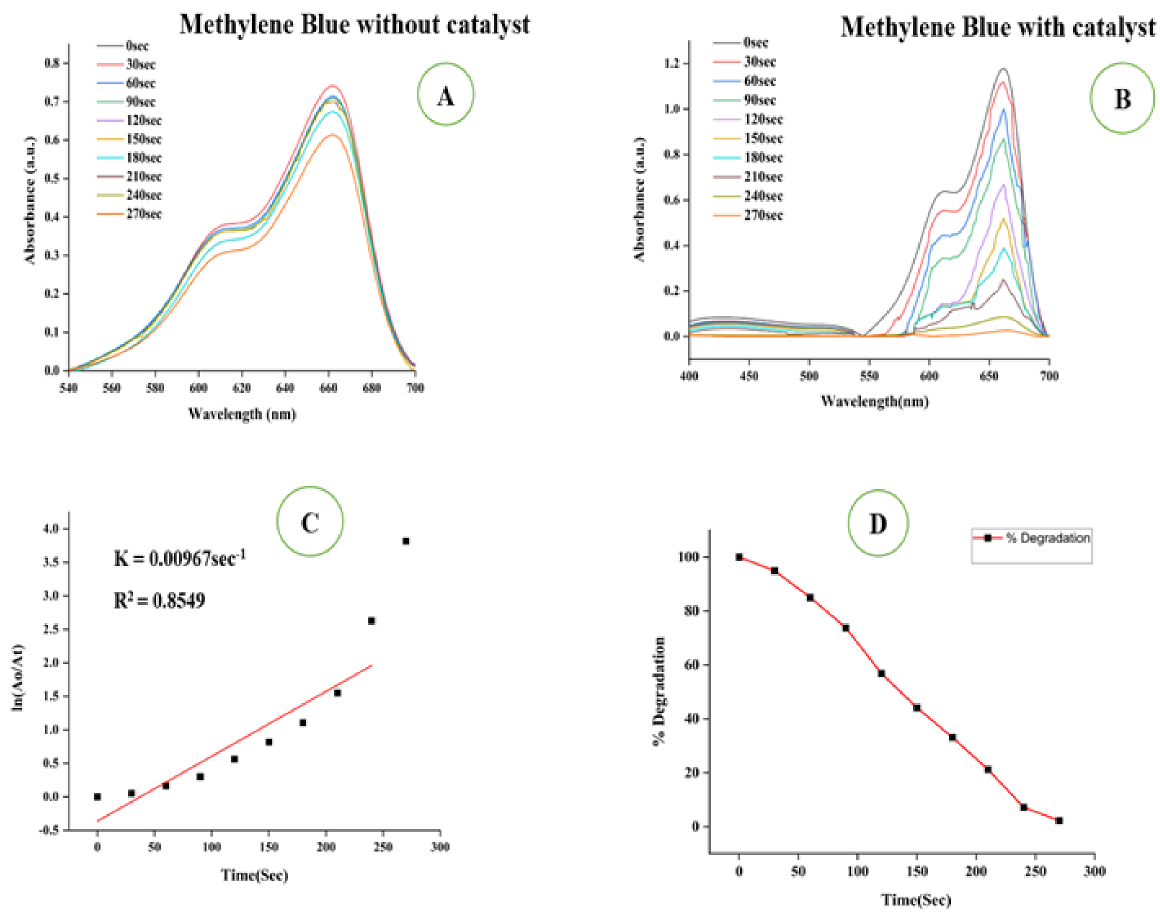

Fig. 9: Ultraviolet-vis spectra showing degradation of (A) Methylene blue dye without catalyst, (B) Methylene Blue dye with catalyst, (C) first order kinetics of catalytic reduction (D) percentage of degradation with NiONPs.

to the disruption of the dye's extensive conjugated structures, primarily through the hydrogenation of functional groups such as -N=N- (azo groups) and -C≡N- (cyano groups). During catalysis, the interaction between the catalyst and the reactants modifies the reaction pathway, effectively reducing the activation energy required for the reaction. This reduction in activation energy significantly enhances the reaction rate, leading to efficient dye degradation. To monitor the degradation process, a UV–vis spectrophotometer was employed to analyze the pure dye solutions within the range of 200 to 800 nm. This analysis permitted the determination of the maximum absorbance wavelength (λmax) for each dye. Rhodamine-B (Rh-B) exhibited a λmax at 552 nm, while Methylene Blue displayed its λmax at 662 nm. The absorbance intensity at these wavelengths was used as a key indicator to track the progress of the degradation reactions, providing insight into the effectiveness of the catalytic process. At the utmost absorption wavelengths of Rhodamine B ($R^2 = 0.9326$), and Methylene blue ($R^2 = 0.8549$), In (Ao/At) versus time has a strong linear relationship, showing that the process follows quasi-first order kinetics shown in Fig. 8(c) and 9(c) with reaction rate constant 0.0170 and 0.0096 s^{-1} respectively. Nickel oxide nanoparticles showed strong catalytic activity, reducing Rh-B dye by 97.71% Fig. 8(d), while methylene blue dye by 90% in 270s Fig. 9(d).

Conclusion

This research outlines the environmentally friendly synthesis of NiONPs under mild conditions, utilizing leaf extracts from *Alternanthera sessilis* as both stabilizing and capping agents. The aqueous extract of *A. sessilis* is rich in phenolic compounds, with a concentration of approximately 47.65 ± 0.006 µg/mL, which are crucial for the reduction and formation of nickel oxide nanoparticles. The green-synthesized nickel oxide nanoparticles were characterized using various techniques, including Ultraviolet-Visible spectroscopy, X-ray Diffraction, FTIR, and SEM. The optical properties of the prepared nanoparticles were analyzed through PL measurements at different stages of the synthesis. The nanoparticles effectively inhibited the growth of the gram-positive bacteria *Bacillus subtilis* and the fungus *Candida albicans*, indicating their potential as antibacterial and antifungal agents. The synthesized NiONPs exhibited significant catalytic activity in degrading organic dyes, such as rhodamine-B and methylene blue, using sodium borohydride ($NaBH_4$) as a dropping agent. The remarkable catalytic performance of NiONPs can be attributed to their high surface area-to-volume ratio, which provides more active sites for reactant molecules to interact. This highlights their potential usefulness in treating industrial wastewater.

The conclusion accentuates the multifunctional nature of NiONPs synthesized from the leaves of *A. sessilis*. These nanoparticles possess antimicrobial and antifungal properties, as well as catalytic activity, making them promising for both environmental and biomedical applications. Additional research into the catalytic stability of these nanoparticles could enhance their value for the elimination of organic pollutants and impurities from manufacturing effluents.

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Author Contribution

Shivani R. Sharma (Conceptualization, designing the experiments, Green Synthesis, biological activity assays, data collection, analysis and interpretation, writing-review and editing), Nilima M. Dhote (provided critical guidance in the experimental design and methodology)Mamta S. Wagh (Conceptualization, formal analysis, Data curation, visualization, Supervision, writing-original draft).

Conflict of interest

The authors declare that there are no competing interests associated with this article's release.

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