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Nickel Oxide Green Synthesized Nanoparticles from Plant Leaves and Its Antimicrobial, Antioxidant Activity and Catalytic Activity in Organic Transformation

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Abstract

Benzimidazole derivatives are an important class of compounds known for their wide range of biological and pharmaceutical applications. In the present study, nickel oxide nanoparticles (NiO NPs) were synthesized and employed as an efficient catalyst for the preparation of benzimidazole derivatives. The synthesized NiO NPs were characterized using XRD, FE-SEM, EDX, UV–Vis and FT-IR techniques. The catalyst promoted the reaction of o-phenylenediamine with various aromatic aldehydes under controlled conditions, providing the desired benzimidazole derivatives in a convenient manner. The synthesized products were monitored and assessed for purity by thin-layer chromatography (TLC) and melting-point, while the catalyst could be easily recovered for further use.

NiO NPs was tested for its antimicrobial activity against S. aureus, B. subtilis, E. coli, K. pneumoniae, and A. niger using the disc diffusion method. No visible zone of inhibition was observed around the NiO NPs discs for any of the tested microorganisms, and the inhibition-zone diameter was recorded as 0 mm in each case. In comparison, the positive controls showed clear zones of inhibition, while DMSO did not show any inhibitory effect. These results indicate that NiO NPs did not exhibit detectable antimicrobial activity against the tested bacterial and fungal strains under the experimental conditions employed. In addition, the NiO NPs demonstrated notable antioxidant activity (85.07%), approaching that of ascorbic acid. Overall, the findings highlight the potential of NiO NPs as a recyclable catalyst and their biological applications.

Keywords: Green synthesis, Nickel oxide, nanoparticles, catalyst, benzimidazole, antioxidant, antibacterial, antifungal

1. Introduction

Nanoparticles are utilized in a wide array of biological and nonbiological applications owing to their exceptional physical and chemical characteristics, distinctive morphology, dimensions, form, crystalline structure, surface area-to-volume ratio, and electro-optical, magneto-optical, chemical, and mechanical attributes. The green synthesis of NPs in the presence of plant extracts, microbes, and enzymes is currently gaining popularity [1]. Scientists and researchers are becoming interested in green synthesis of NPs because of its quick, affordable, environmentally beneficial, and sustainable method [2]. By trapping the metal ions in the amylose helix at specific

locations, the plant extract functions as a capping agent through a coordination action [3]. By acting as a biotemplate, the roughness of plant leaves and petals regulates nanoparticle size and prevents particle aggregation [4] Ni (0) nanoparticles are used as catalysts in hydrogen auto-transfer and transfer hydrogenation reactions, low-temperature carbon monoxide and propane oxidation, stainless steel surface diffusion bonding, nitrobenzene hydrogenation, and supercapacitor electrode materials [5,6]. By inhibiting adsorption or detachment of the active materials, these materials' surface characteristics improve the contact surface area of the conducting carbon materials, which helps to boost the conductivity of the system. It is therefore preferable to replace other carbonous elements from plants with these materials in order to encourage metal oxide as an electron conductor and to decrease the agglomerations of the nanomaterials during cycles [7-9].They also have several catalytic and therapeutic uses and exhibit antioxidant, anticancer, cytotoxic, antifungal, antibacterial, enzyme inhibition, and remediation characteristics [2-13].Because of their distinct surface area, capacity to release and adsorb metal ions, and cytotoxic effects, they are also used therapeutically as biomedicines [14]. Here, we introduce a green synthesised NiO NPs catalyst from Syzgium cumini plant leaves extract for the eco-friendly one-pot synthesis of derivatives of substituted benzimidazoles. TLC and Melting point were used to confirm benzimidazole derivatives. Using XRD, FE-SEM, EDX, UV, FT-IR the catalyst NiO NPs has been carefully examined to comprehend its physio-chemical. It was discovered that the resulting material (NiO NPs catalyst) can be recovered and recycled up to six times without appreciably reducing the yield since it is stable under the reaction conditions.The biological activities of the green-synthesized NiO NPs, including antibacterial, antifungal, and antioxidant properties, were also investigated.

2. Experimenital

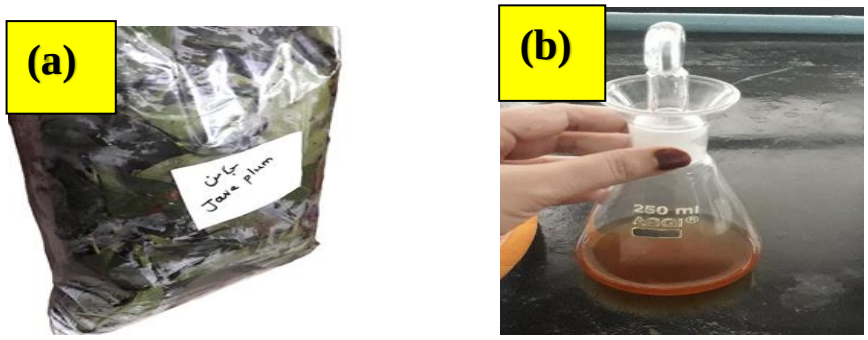
2.1. Material and Methods:

2.1.1. Materials:

Fresh Syzgium cumini leaves were collected in the Marathwada region of Maharashtra, near Aurangabad, India. The leaves were cleaned, shade dried, and coarsely powdered using a blender. The powder was stored in sealed containers at 28 ± 2 °C and sun-protected until ready to use. Sigma-Aldrich of India supplied $NiSO_4 \cdot 6H_2O$, sodium hydroxide, distilled water,99% n-hexane, 99% EtOH, and 99% ethyl ethanoate. Fine Chem Limited provided 99% pure O-phenylenediamine and various substituted aldehydes.

2.1.2. Preparation of Leaf Extract:

Distilled water was used to regularly clean and debase the Syzgium cumini leaves.Ten grams of the cleaned leaves were made into a powder using a blender after being dried in the shade. To extract the plant components, the powder was combined with 100 mL of double-distilled water and heated to 70 degrees Celsius for one hour. The extract was then collected for use as a common lessening and capping operator in the nanoparticle blend after being sorted using Whatman No. 1 filter paper. **(Fig.1)**



(a) (b)

Fig. 1: Syzgium Cumini plant (a) Fresh Plant Leaves (b) leaves Extract

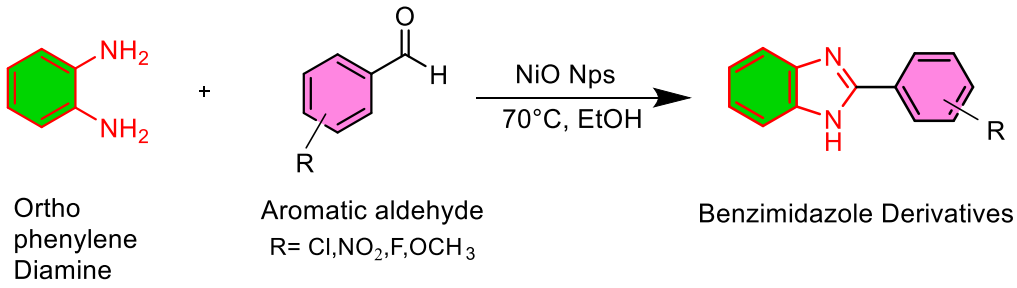
2.1.3. Green Synthesis of NiO Nanoparticles:

Syzgium cumini leaf extract was used in a modified green approach to create Nickel oxide

nanoparticles (NiO NPs). After preparing a 0.1 M $NiSO_4 \cdot 6H_2O$ solution in 80 mL of distilled water and blending it for 30 minutes, subsequently, 10 mL of the prepared leaf extract was added slowly to the nickel salt solution with continuous stirring. The mixture was continuously blended at 500–700 rpm while being heated to 70 °C for two hours. After using 0.01 M NaOH to balance the pH to 9–10, the liquid was centrifuged for 10 minutes at 8000 rpm. The substance underwent washing, drying at 70–80 °C, powdering, and calcining at 400–500 °C and black colour powder is obtained. To aid in characterisation and catalytic study, the obtained NiO NPs were stored in a sealed shut holder **(Fig. 2)**.

2.1.4. General technique for NiO Nano Particle catalyzed benzimidazole synthesis:

Scheme 1:



For the synthesis, orthophenylene diamine (1 mmol) and the corresponding aldehyde (1 mmol) were mixed in EtOH, followed by the addition of NiO NPs (2.5%, 0.030 g). The solution was warmed to 70 °C and blended until the reaction was complete, which was monitored by TLC using n-hexane and ethyl ethanoate (7:3, v/v). After cooling, 10 mL of hot ethanol was added to assist catalyst separation via filtration **(Scheme 1)**.

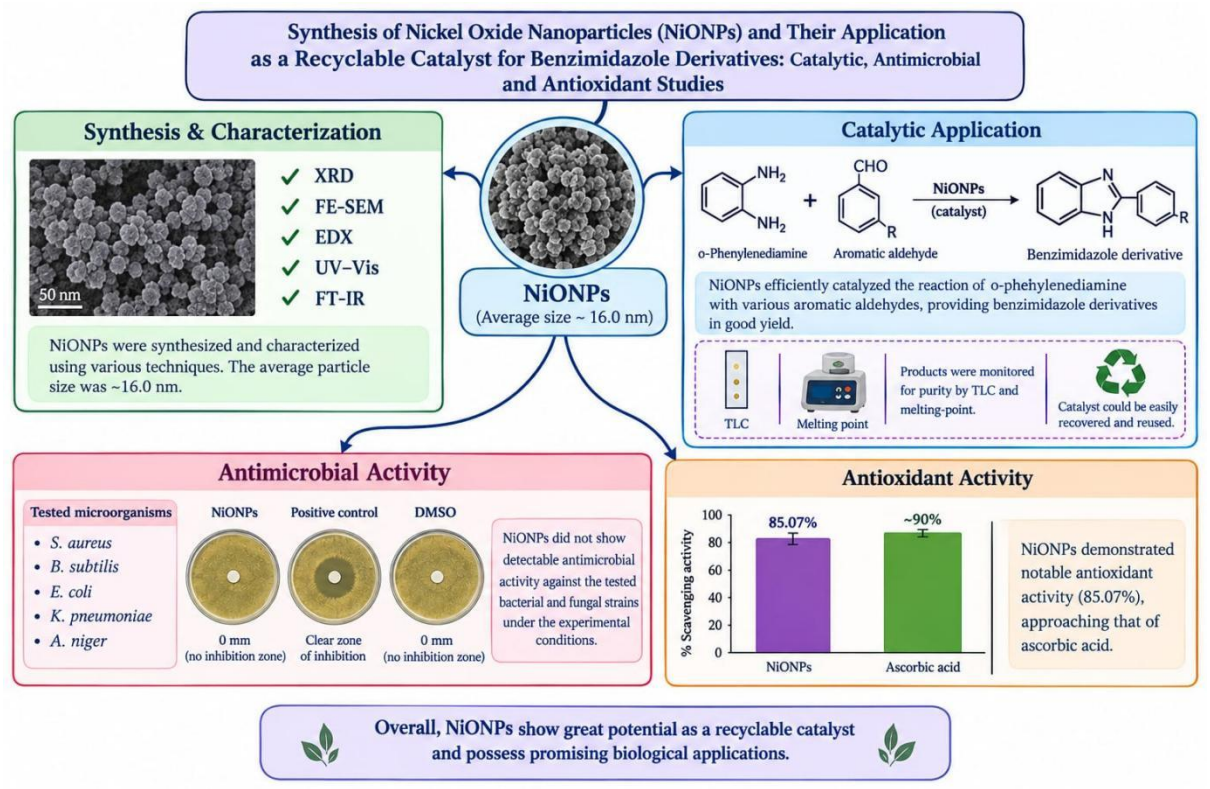


Fig. 2: Graphical abstract of Nickel oxide Green synthesised nanoparticles from Syzgium cumini plant leaves and its antimicrobial, antioxidant activity and catalytic activity in organic transformation

Table 1: Benzimidazole derivatives catalysed by NiO Nano Particles

Entry	R	Yield %	Time (Min)	M.P
J1	2,4-Cl	80	10	315-319°C
J2	2-NO2	65	26	209-211°C
J3	4-NO2	82	18	312-314°C
J4	4-F	72	20	225-229°C
J5	2,3,5-OCH3	70	8	259-261°C

The filtrate was recrystallized from ethanol to get the desired crystalline benzimidazole precursor product **(Table 1)**. Their synthesis was validated via Thin Layer chromatography and melting point analysis, with selected samples compared to reported values.

3. Result and discussion:

3.1. IR of NiO nanoparticles:

FTIR spectroscopy was used to analyze the various functional groups present in the Syzgium cumini leaf extract and to confirm the formation of NiO NPs. The FTIR spectra of the Syzgium cumini leaf extract and NiO NPs recorded over the range of 4000–400 cm^{-1} **(Fig. 3)** The peaks found at 1118 cm^{-1} attributed to the C-O group indicate the presence of alcohols and carboxylic acid groups involved in the nanoparticle formation [15].The CO_2 stretching vibration mode displayed the strong absorption band at 2357cm^{-1}, possibly originating from aerial CO_2 or CO_2 trapped within the nanoparticle grains. Rapid carbon dioxide and water adsorption suggests a high surface area for the material [16] The plant extract's aromatic group demonstrated the broad band at 1429 cm^{-1}. Stretching frequencies in of metal and oxygen 418 cm^{-1} are attributed to Ni-O bonds.

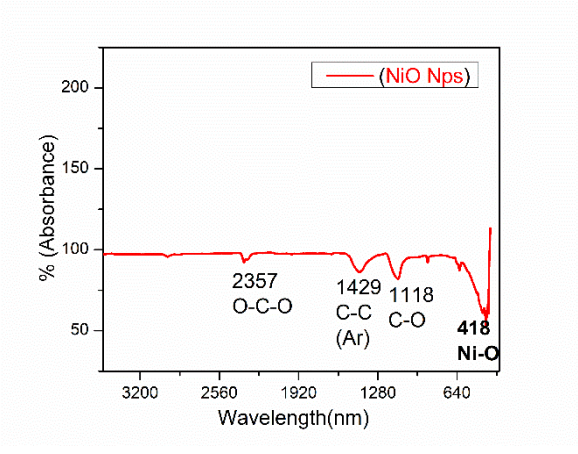


Fig. 3:IR of synthesised NiO NPs

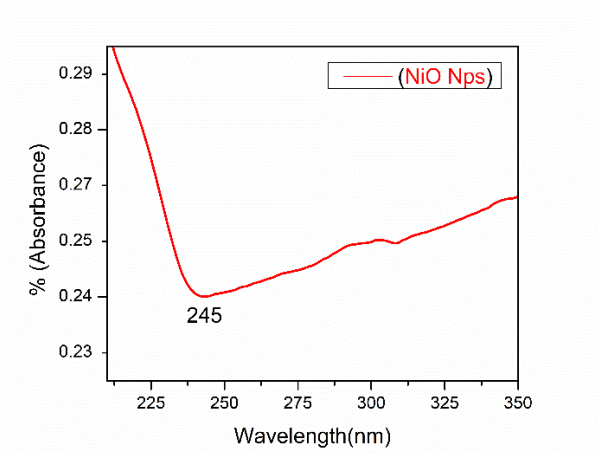


Fig. 4:UV of synthesised NiO NPs

3.2. UV of NiO nanoparticles:

UV visible spectroscopy was used to analyze the various functional groups present in the leaf extract and to confirm the formation of NiO NPs.UV–Visible analysis conducted between 200 and 1000 nm in wavelength. **(Fig. 4)** illustrates the nickel oxide nanoparticles UV–Visible spectra, a distinct peak of absorption is observed at 245 nm wavelength.

3.3. XRD of NiO nanoparticles:

(Fig. 5) shows the X-ray diffraction pattern of NiO NPs scanned from 35° to 60° at an angle of 2θ (2 theta). The diffraction peaks at 36.40°, 47.74°, 56.76°correspond to the (111), (200), (220) planes.This pattern confirms the crystallinity of green-synthesised NiO nanostructures. All of the reflections in the XRD pattern can be indexed to face-centered cubic phase NiO (JCPDS card no. #47-1049). [17,18,19].

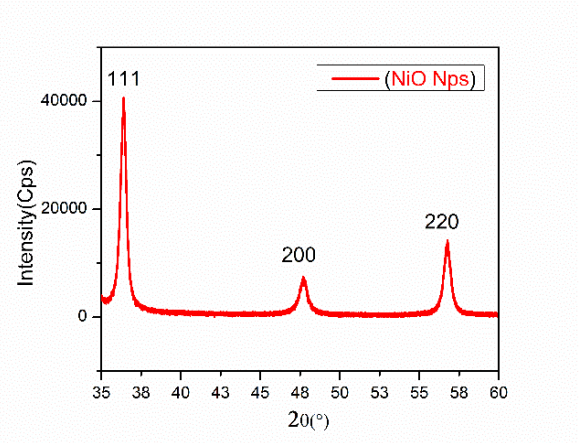


Fig. 5: XRD of Synthesised NiO NPs

3.4. FE-SEM and EDX of NiO nanoparticles:

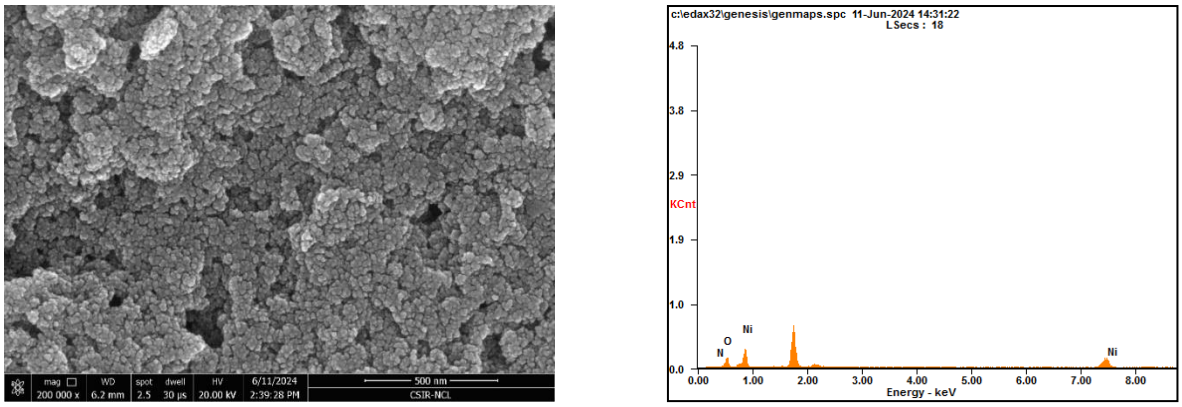


Fig. 6:(A) FE SEM of Synthesised NiO NPs **(B) EDX of synthesised NiO NPs**

Scanning electron microscopy was used to examine the surface morphology of pure NiO nanoparticles samples at 500nm magnifications **(Fig. 6 A)**. The SEM analysis shows that the produced nanoparticles are well-dispersed, cubical, and extremely crystalline (<10 nm), consistent with previous studies [20].The produced nanoparticles are agglomerated due to aggregation or overlapping of smaller particles, resulting in a cluster of nanoparticles.[21] To know the elemental composition of the biosynthesized NiO NPs, the EDX analysis was employed. The Nickel and oxygen signals of NiO NPs are confirmed by the EDX elemental mapping and the distribution of Nickel aggloromation is displayed in **(Fig. 6 B)** The elemental analysis of the nanoparticle revealed 50.46% Nickel, 42.95% oxygen and Nitrogen 6.60% demonstrating the maximum level of purification for the produced nanoparticle in **(Table 2)**.

3.5. Biological Application of NiO nanoparticles:

3.5.1. Antimicrobial activity of synthesised NiO NPs:

The antimicrobial activity of N_2 (NiO NPs) was studied by the agar disc diffusion method against four bacterial strains and one fungal strain. **(Fig. 7)** NiO NPs was tested for its antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, and Aspergillus niger using the disc diffusion method. No visible zone of inhibition was observed around the NiO NPs discs for any of the tested microorganisms, and the inhibition-zone diameter was recorded as 0 mm in each case. In comparison, the positive controls showed clear zones of inhibition, while DMSO did not show any inhibitory effect. These results indicate that NiO NPs did not exhibit detectable antimicrobial activity against the tested bacterial and fungal strains under the experimental conditions.

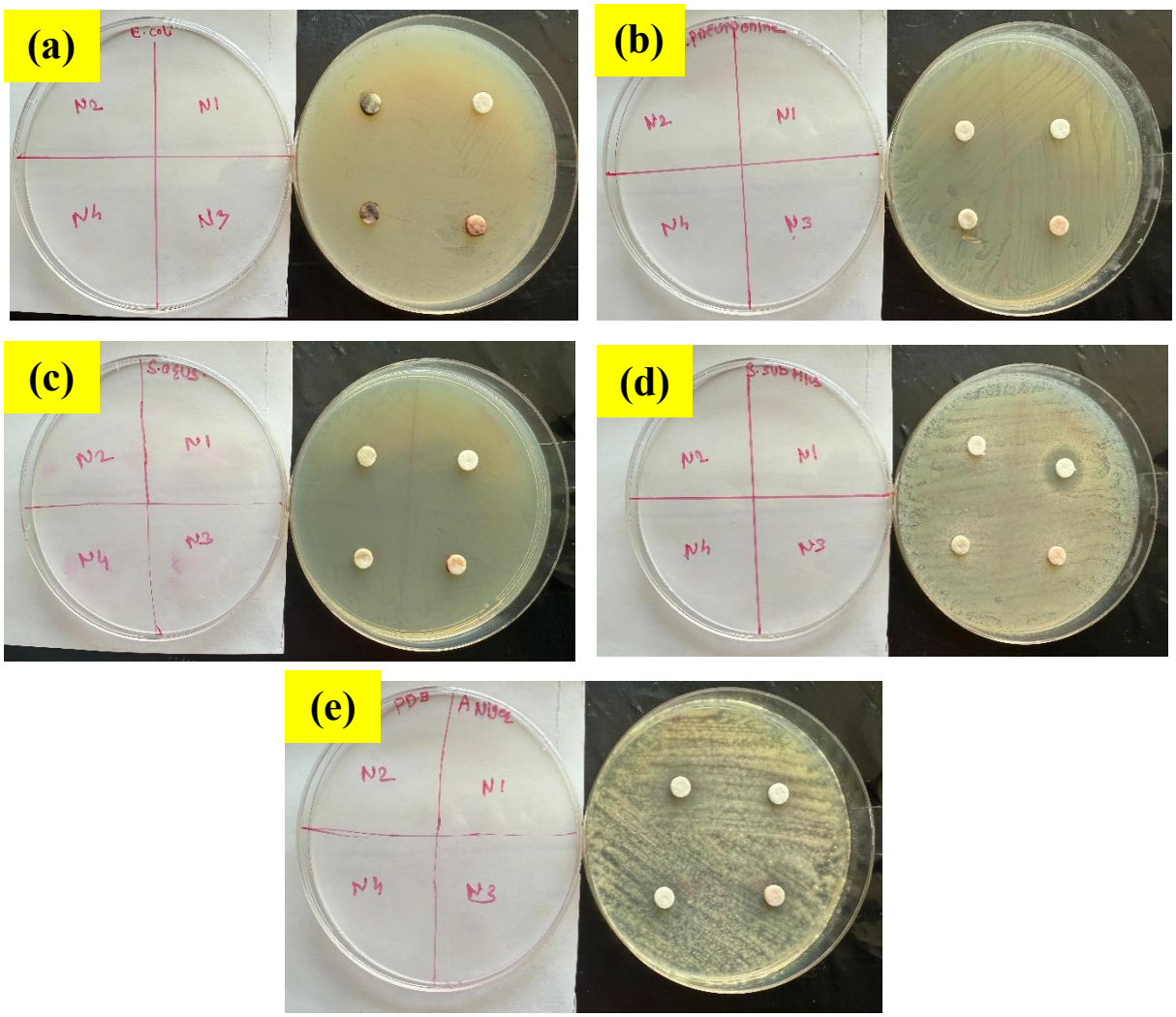


Fig. 7: Antimicrobial Activity of Green synthesised NiO NPs (N2) (a) Escherichia coli(b)Klebsiella pneumoniae (c) Staphylococcus aureus (d) Bacillus subtilis (e)Aspergillus Niger

Table 2: EDX analysis of synthesized NiO NPs percent composition

Element	Wt%	At%
NK	06.60	11.74
OK	42.95	66.86
NiK	50.46	21.41
Matrix	Correction	ZAF

3.5.2. Antioxidant Activity of synthesised NiO NPs:

The DPPH radical scavenging assay demonstrates a concentration-dependent antioxidant activity for the standard, sample code (N2) NiO NPs. The percentage inhibition increased progressively with increasing concentration from 20 to 100 µg/mL, indicating enhanced free-radical scavenging capacity at higher concentrations. The standard showed the highest activity, increasing from approximately 25% inhibition at 20 µg/mL to 82% at 100 µg/mL. **Table 3** shows NiO NPs exhibited greater antioxidant activity. Overall, NiO NPs demonstrated appreciable DPPH radical-scavenging activity, particularly at higher concentrations, although their activity remained lower than that of the standard. The results suggest that NiO NPs possesses better antioxidant potential and the activity appears to be dose dependent.

Table 3: The DPPH radical scavenging assay of synthesised NiO NPs

Sample Code	Concentration (µg/ml)	Absorbance at 510nm				% Inhibition	IC50 (µg/ml)
		Test1	Test 2	Test 3	Mean		
Control	-	1.93	1.93	1.93	1.93	-	
Standard	20	1.45	1.42	1.40	1.42	26.42%	59.17
(Ascorbic Acid)	40	1.31	1.29	1.33	1.31	32.12%	
	60	0.95	0.97	0.93	0.95	50.77%	
	80	0.82	0.85	0.79	0.82	57.51%	
	100	0.35	0.32	0.32	0.33	82.90%	
N_2	20	1.77	1.75	1.71	1.74	9.67%	85.07
	40	1.53	1.52	1.50	1.52	21.42%	
	60	1.29	1.33	1.35	1.32	31.43%	
	80	0.99	1.02	1.06	1.02	46.98%	
	100	0.79	0.80	0.79	0.79	58.89%	

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3.5.3. Recycling study of catalyst:

Under optimum conditions, benzaldehyde and o-phenylenediamine were employed to test the NiO NPs catalyst's reusability. After completion, the catalyst was separated by adding 10 mL of Ethanol and filtration, then cleaned with dichloromethane and dried for three hours at 50 °C before being reused. The catalyst produced a 91% yield in the first cycle, and yields maintained over 89% for the next six. [22]

4. Conclusion:

In the present study, nickel oxide nanoparticles (NiO NPs) were successfully synthesized and characterized using XRD, FE-SEM, EDX, UV–Vis, and FT-IR techniques. The nanoparticles showed an average particle size of approximately 16.0 nm and demonstrated good catalytic efficiency in the synthesis of benzimidazole derivatives from o-phenylenediamine and different aromatic aldehydes. The reaction products were readily monitored for purity using TLC and melting-point analysis. An important advantage of the synthesized NiO NPs was their easy recovery, indicating their potential for reuse as a heterogeneous catalyst.

The biological studies showed that NiO NPs did not produce any detectable antimicrobial activity against the tested bacterial and fungal strains under the experimental conditions, as no inhibition zones were observed. However, the nanoparticles exhibited considerable antioxidant activity, reaching 85.07%, which was close to that of ascorbic acid.

Overall, the results suggest that the synthesized NiO NPs are a useful and recyclable catalyst for benzimidazole synthesis and also possess promising antioxidant properties, making them worthy of further investigation for potential biological applications.

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Conflict of Interest:

The authors declare that there is no conflict of interest.

Declaration of AI-assisted technologies:

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

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