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Green Synthesis of Nickel Oxide Nanoparticles using *Vitex negundo* Leaf for Antibacterial and Anti-inflammatory Activities

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This study addresses the crucial need for an environmentally sustainable method by synthesizing Nickel Oxide nanoparticles (NiO NPs) by using *Vitex negundo*(VN) leaf extract as a natural stabilizing and reducing agent. As the demand for green nanoparticle synthesis continues to increase and innovative approaches are essential to reduce reliance on harmful chemicals and high energy consumption. The synthesis of NiO NPs process was carried out at room temperature, followed by annealing at 500°C and 700°C to enhance the nanoparticles' properties respectively. X-ray diffraction (XRD) analysis revealed improved crystallinity at higher temperatures, with crystalline sizes of 20.91 nm at 500°C and 32.61 nm at 700°C. These findings underscore the potential of using plant extracts of green synthesis, offering a sustainable pathway for producing high-quality nanoparticles and contributing to the advancement of eco-friendly nanotechnology. The UV-Vis spectrum showed an energy band gap of 3.38 eV. SEM analysis revealed spherical particles with some agglomeration, while EDX confirmed the elemental composition. FTIR analysis identified functional groups in the sample annealed at 500°C. Antibacterial testing using the agar well diffusion method demonstrated activity against *B. subtilis, S. aureus, E. coli, and P. aeruginosa.* Additionally, the nanoparticles exhibited significant anti-inflammatory activity, highlighting their potential for biomedical applications.

Keywords: Biomedical Applications, Eco-Friendly, Nanotechnology, Nickel Oxide Nanoparticles, *Vitex negundo* leaf

Since ancient times, the human population of the planet has faced numerous significant health issues. By producing numerous infectious diseases, microorganisms like viruses, bacteria, fungi and pathogens have contributed to the emergence of a pandemic environment. In the context of substantial research for emerging nanotechnology that can combat pathogenic disorders because of the rapidly expanding infectious diseases[1]. Nanoparticles can be made using a range of methods, such as chemical, physical, biological, and hybrid processes. However, the green synthesis method for producing nanoparticles is environmentally beneficial as it reduces toxicity and yields safer substances[2]. The discipline of "Green Chemistry" has seen the emergence of biological methods for the cost-effective synthesis of metallic NPs, including Nickel Oxide NPs against bacterial infectious diseases. The green synthesis of Nickel Oxide NPs has been accomplished recently, and there is growing attention in the synthesis of Nickel Oxide NPs from several additional medicinal plants for various biological applications[3]. The use of plant extracts to synthesize metal oxide nanoparticles has several advantages, including the absence of harmful byproducts, which has piqued interest in exploring new types of plant extracts for Nickel Oxide NP production[4]. Current assessments on environmentally benign nanoparticle synthesis have verified the approaches' great potential for producing metal oxide on a large scale[5]. Numerous products, devices and different pest management methods use Nickel Oxide NPs, including catalysts, fuel cells, dye-sensitized photocathodes, electrochromic films, battery electrodes, photo-electron devices, ion storage materials, gas sensors, magnetic materials, thermoelectric materials, anticancer, cytotoxic action, non-enzymatic glucose sensors, *etc*[6]. Antibacterial activity of Nickel Oxide NPs is also present against gram-positive and gram-negative microorganisms[7]. Green-synthesized nickel oxide nanoparticles are proving invaluable in various biological applications. Phytogenic synthesis of Nickel Oxide nanoparticles used *Rhamnus triquetra* leaf extract it is used in

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multiple biological applications such as antioxidant, antibacterial, Antifungal activity, Antileishmanial Potentials and Anticancer[8]. Green synthesis of Nickel Oxide nanoparticle used *Tamarix serotina* leaf extract it is used catalytic activity[9]. Green synthesis Nickel Oxide nanoparticles used *Cassia auriculata* plant leaf extract it is used in biological applications such as antimicrobial activity[10]. Green synthesis of Nickel Oxide nanoparticle *Euphorbia heterophylla* leaf extract it is used for biological applications such as Anticoagulant and antibacterial activities[11]. Green synthesis of Nickel Oxide NPs used for *M. Oleifera* leaf extract used numerous biological applications such as photocatalytic activity and antibacterial activity[12,13]. Stevia leaf extract is used in the environmentally friendly manufacture of Nickel Oxide nanoparticles, and their*in vitro* antioxidant and antimicrobial properties are assessed[14]. Since ancient times, the plant parts like leaves, stems, flowers, and bark have been used as natural medicines. Wisely, the plant proteases enzymes with healing, antimicrobial, and anti-inflammatory properties that aid digestion and wound healing[15-18]. Plant extracts offer eco-friendly solutions through nanotechnology and biomedicine, enabling the green synthesis of nanoparticles with antibacterial, antioxidant, and anti-inflammatory effects for safer applications[19-22]. This study uses *Vitex negundo* leaf extract to synthesize Nickel Oxide nanoparticles with antibacterial activity. Known for its bioactive compounds, including flavonoids, polyphenols, and terpenoids has valuable disease-preventive properties.*Vitex negundo* is a key player in both the COVID-19 epidemic and chronic health problems. Natural traditional systems use *Vitex negundo* Linn, which has several medicinal uses[23-25]. Major chemical contents include limbolol, linalool, α-Elemol, α-Farnesene, Aromadendrene, Sabinene, Terpinen-4-ol, -terpinene, Oct-1-en-3-ol, Globulol, and numerous terpenes that have been shown to have good therapeutic qualities[26-28]. *VN* is used to treat rheumatism, catarrhal fever, sinusitis, dysmenorrhea, and syphilitic skin condition. In Unani medicine, the seeds of the plant are used as an aphrodisiac and to treat swellings. Eating the VN fruit is recommended by Chinese medicine to treat puffy eyes, arthritis, and migraines[29,30].

Experimental work

Preparation of plant extract

Fresh, healthy leaves of *Vitex negundo* were collected and washed thoroughly with running tap water, followed by rinsing with distilled water to remove dust particles and reduce any residual contaminants. 30 g of fresh *Vitex negundo* leaves were combined with 1000 mL of deionized water which were then boiled for 3 h. The resulting extract was filtered through the Whatman filter paper[12,13].

Synthesis of NiO nanoparticles

The precursor salt solution was made in the beaker by dissolving 2.24 g of nickel chloride hexahydrate in 50 mL of distilled water. The prepared plant extract was then added 20 mL increments at a time. The reaction combination was heated to 60°C for 30 min while being stirred[14]. Finally, a powder sample is obtained this sample is used for further characterizations[1,10].

Characterization

The structural analyses of NiO NPs were carried out by Powder X-ray diffraction (PXRD) (Model-Smart Lab SE X-Ray; Make- Rigaku, Japan).Optical characterization was performed using a UV-Vis spectrophotometer (model UV-1800, serial number A11635581319) to analyse the absorption spectrum. It was recorded with the wavelength range 200 nm to 1100 nm. The morphological structure was examined by the Carl Zeiss Supra (SEMEVO18). Energy Dispersive X-ray – spectrometer Quantax 200 X Flash used for elemental compositions. Functional group identification was using FTIR IR Tracer 100 spectra were documented in the range of 4000–400 cm^{-1}.

Results and Discussion

Physical Characterizations of the NiO NPs

To synthesize NiO NPs, various physical characterization techniques were investigated. As synthesized at 100°C and annealed at 500°C and 700°C, the *Vitex negundo* leaf extract of the PXRD is used in (Fig.1& 2) to illustrate the green synthesis of NiO nanoparticles.

The miller indices (hkl) planes at (111), (002), (202), (113) and (222) correspond to potential diffraction peaks at 2θ values of 37.51°, 43.54°, 63.10°, 75.58° and 79.60°. The peaks correspond to the average (JCPDS card number 04-0835). For both the 500°C and 700°C annealed samples, the same card was coordinated. The face-centered cubic crystal structure of Nickel Oxide nanoparticles is shown by their diffraction peaks(Fig.3). Debye Scherrer formula has been used to determine the average crystallite size of produced Nickel Oxide nanoparticles:

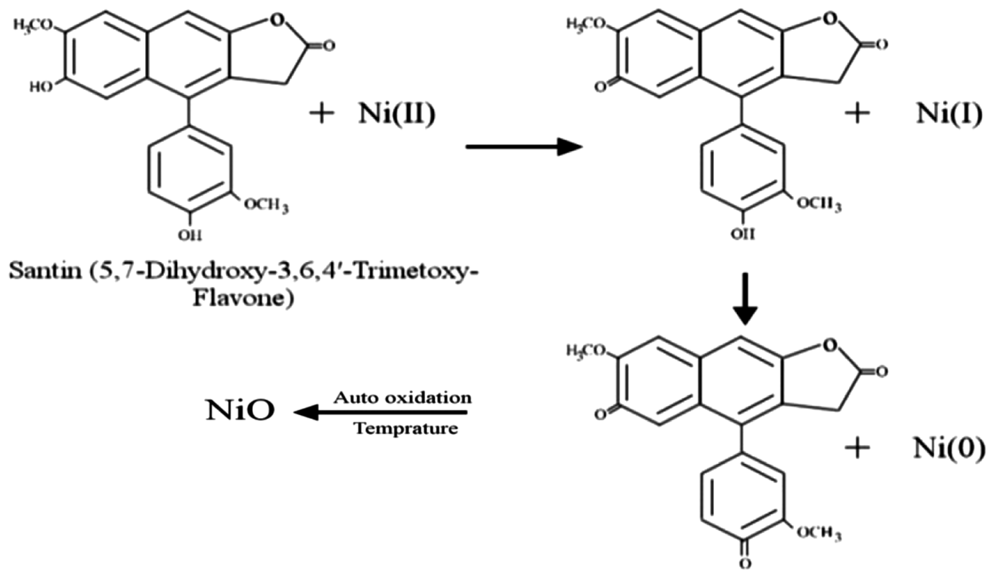

Fig. 1 — Reaction mechanism for the green production of NiO NPs proposed

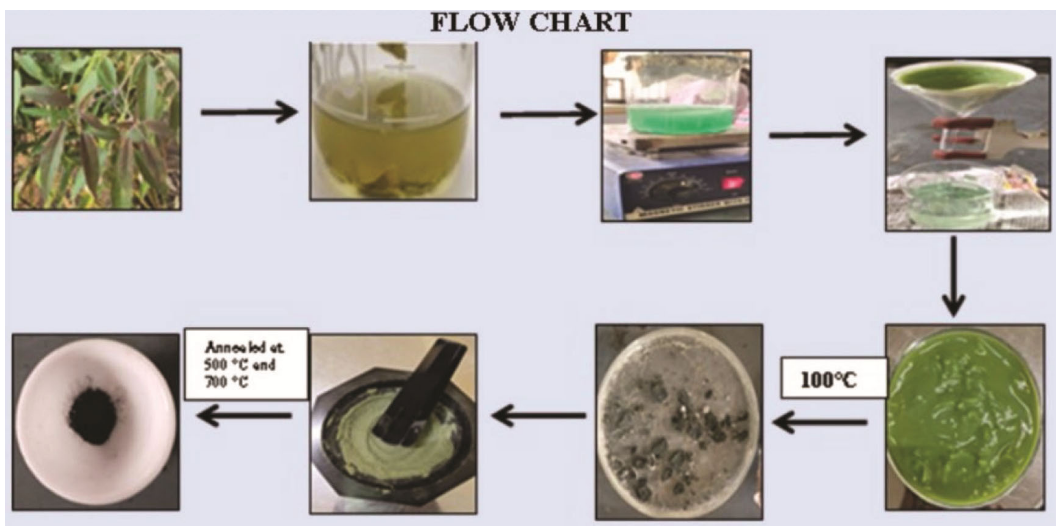

Fig. 2 — Preparation and green synthesis of NiO NPs using *Vn*leaf extract

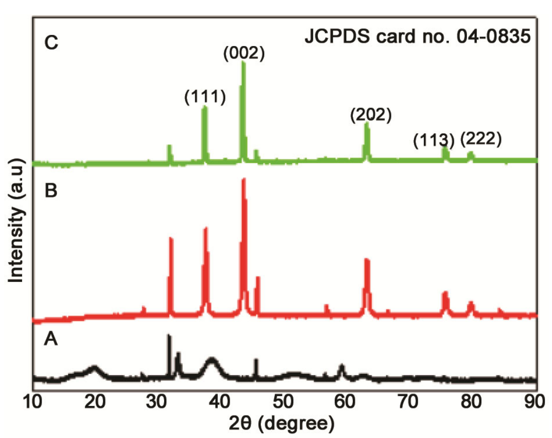

Fig. 3 — The XRD pattern of Nickel Oxide nanoparticles (A) prepared sample; (B) annealed at 500°C; and (C) annealed at 700°C

$$D = \frac{k\lambda}{\beta \cos\theta} \quad \ldots(1)$$

Where D, K and λ represent the crystal size, Shape factor and wavelength of the X-ray. β and θ represent the full width at half maximum intensity of the peak and diffraction angle. And also calculated the lattice constant ‘a’ using the below formula:

$$a = d_{hkl}(h^2 + k^2 + l^2)^{1/2} \quad \ldots(2)$$

Debye-Scherrer equation states, that the typical crystalline sizes of green synthesis NiO NPs are 20.93 nm and 32.61 nm. Calculated particle sizes, interplanar distances and lattice constants of the

Table 1 — Particle size, lattice constant, and interplanar distance of Nickel Oxide NPs at 500°C were calculated

2θ	Hkl	FWHM (β)	d-spacing (Å) Observed	d-spacing (Å)Calculated	Crystal size (D)	Lattice constant (a)
37.51	111	0.37	2.39	2.39	22.42	0.41
43.54	002	0.38	2.07	2.07	22.02	0.29
63.10	202	0.47	1.47	1.47	19.56	0.29
75.58	113	0.45	1.25	1.25	22.26	0.28
79.60	222	0.56	1.20	1.20	18.37	0.29

Table 2 — Particle size, lattice constant, and interplanar distance of Nickel Oxide NPs at 700°C were calculated

2θ	Hkl	FWHM (β)	d-spacing (Å) Observed	d-spacing (Å) Calculated	Crystal size (D)	Lattice constant (a)
37.39	111	0.19	2.40	2.40	42.57	0.41
43.42	002	0.19	2.08	2.08	44.30	0.29
63.03	202	0.33	1.47	1.47	27.81	0.29
75.50	113	0.39	1.25	1.25	25.37	0.28
79.58	222	0.44	1.20	1.20	23.02	0.29

Nickel Oxide nanoparticles are annealed at 500°C and 700°C are shown in (Tables 1 & 2).In Figure3 XRD data shows some additional peaks are present in the 500°C[31]. These peaks indicate some biochemical constituents present in the plant[32,33]. Increase the annealing temperature biochemical constituents would vanished[34].

Absorption and bandgap measurements of prepared samples are shown in (Fig.4) and 4.1 using a UV-Vis spectrophotometer. Larger in size and with a reduced surface area, Nickel Oxide NPs are typically weakly absorbed in the ultraviolet spectrum. On the other hand, Nickel Oxide NPs have a larger surface area and are smaller in size, which allows them to absorb intensely and efficiently absorb photons. The absorbent bandgap of Nickel Oxide semiconductors is responsible for the strong absorption in the UV region that has been observed. One can get the bandgap Eg energy using the tauc equation[35], which is provided by:

$$(\alpha h\nu)^{1/n} = A\,(h\nu - E_g) \quad \ldots(3)$$

Nickel Oxide is verified as a direct semiconductor at 3.38 eV. (Fig. 5 fromA, B, C and D) shows the SEM images of biosynthesized Nickel Oxide NPs annealed at 500°C. SEM analysis shows particles are spherical with agglomeration[10,14].

Peaks of Ni and O were seen in the EDX study (Fig. 6), with corresponding percentages of 63.7% and 18.2%, respectively. The other elements include Cl (8.9%) and Na (9.1%) whose capacity comes from organic substances that encircle the NiO NPs surface[1,31].

The FTIR spectrum of the *Vitex negundo* leaf extract indicates an intense band at ~3425 cm^{-1} for H_2O which plays a significant role in both stabilization and reduction of Nickel Oxide NPs[3]. A

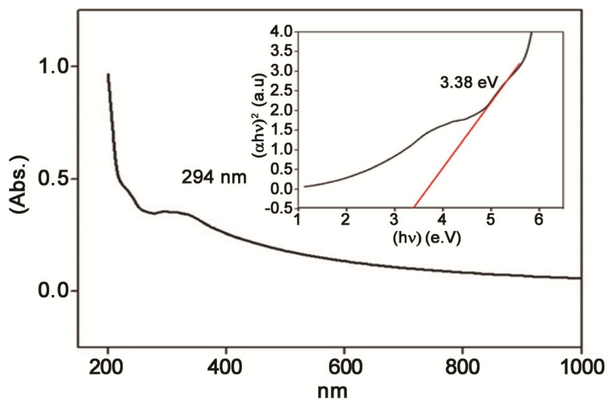

Fig. 4 — Absorption spectrum and energy bandgap of Nickel Oxide NPs for prepared sample

peak of 2981 cm^{-1} (C-H) is present in the FTIR spectrum of aqueous leaf extract[6]. A peak of 1622 cm^{-1} indicates the presence of –OH groups[3]. A peak of 1412 cm^{-1} corresponds to O-H bending present in that spectrum[4]. The deformation vibration of NO_3 peaks at 1360.7 cm^{-1}, indicating stretching for symmetric nitrates[36]. A peak of 1249 cm^{-1} indicates absorption Peaks present in the FTIR spectrum[1]. 1129 cm^{-1} and 1621 cm^{-1} both peaks have links to the C-H and C[2]. A peak of 1030 cm^{-1} indicates the existence of –OH groups in the spectrum[3]. 741 cm^{-1} peak indicates FTIR spectrum of Nickel Oxide falls within the extending frequencies of metal-oxygen[4]. 663 cm^{-1} shows the nickel oxygen bond stretching vibrations[6]. A stretching vibration of (Ni-O-H) is indicated by a peak at 563 cm^{-1} in that spectrum[36].Here, (Fig. 7) shows the FTIR spectrum.

Biological application of the NiO NPs

Antibacterial Activity

From (Fig. 8A & B) illustrates how the antibacterial activity of Nickel Oxide NPs was

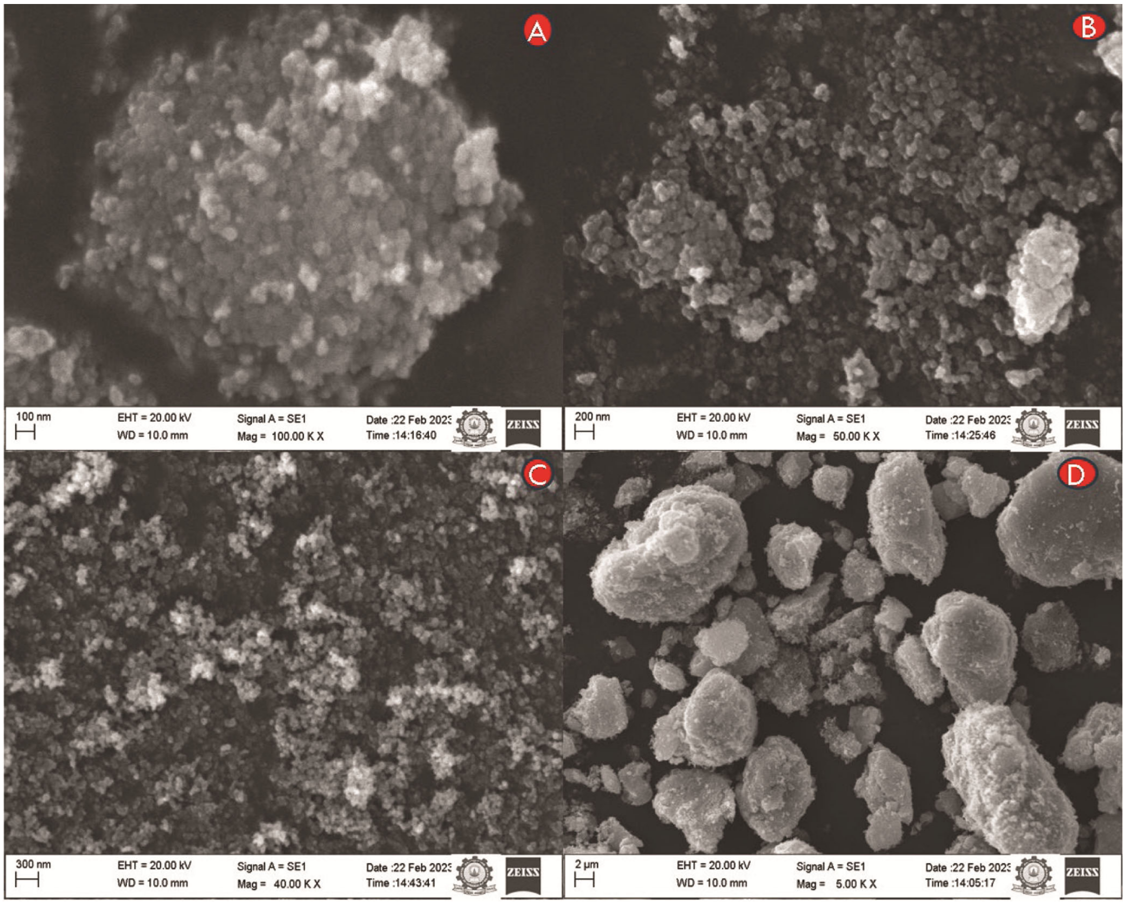

Fig. 5 — (A) represents the 100 nm magnification; (B) represents the 200 nm magnification; (C) represents the 300 nm magnification; and (D) represents the 2 μM magnification

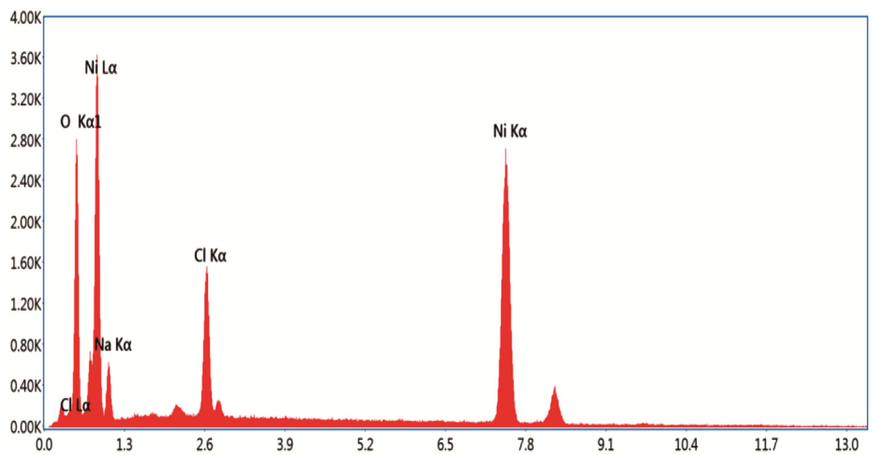

Fig. 6 — EDX graph shown in green synthesis of NiO nanoparticle using *Vn* leaf extract

annealed at 500°C. Various bacterial cultures were used to explore these activities.

(1) Gram positive bacteria – *S. aureus and B. subtilis*

(2) Gram negative bacteria – *P. aeruginosa and E. Coli*

With a rise in NPs at varied concentrations (125 μg, 250 μg, 500 μg, and 1000 μg), the antibacterial potential increased[2]. For the antibacterial assay, four distinct concentrations of NiO NPs were compared with commercial antibiotic streptomycin as the reference (Table3). Particle sizeinfluences

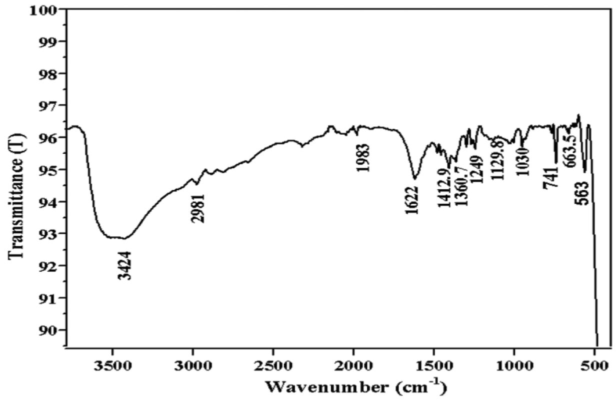

Fig. 7 — FTIR graph in green synthesis of NiO nanoparticle using *Vn* leaf extract

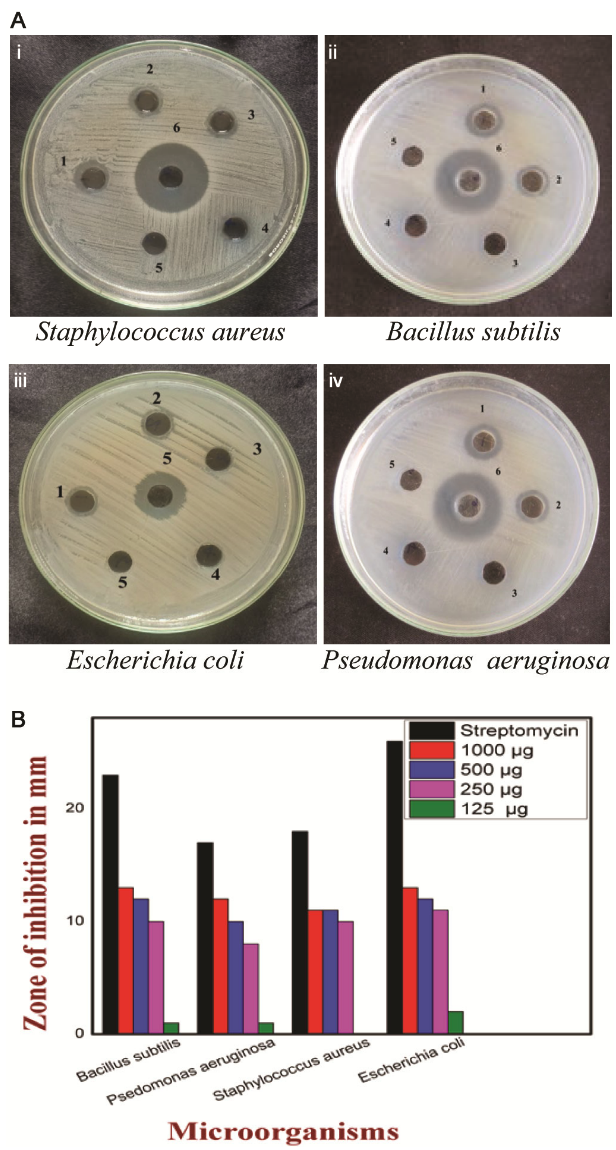

Fig. 8 — (A) (i-iv) Showed the antibacterial activity present in the green synthesis of NiO nanoparticles by *Vn* leaf extract; and (B) Antibacterial activity studies

antibacterial activity in a significant way. Antibacterial outcomes can vary depending on the application of various plant components, herbal extracts, and solvents approach, environmental microorganisms, the area where the plants are grown and the plant harvesting schedule[37].The bio-synthesized Nickel Oxide NPs have a great surface area to volume ratio, a very small size, and excellent stability. The majority of research has demonstrated that when particle size decreases, antibacterial activity rises[8]. The negatively charged bacterial cell membrane and the positively charged nickel ions may have interacted electrostatically to produce the NiO nanoparticle. When Nickel ion (Ni^{2+}) out of NiO nanoparticles is released, it enters the cell wall, damages DNA, proteins, and mitochondria, and disrupts electron movement, which kills the cell[13].

According to this work, the antibacterial activity results shown, *B. subtilis,P. aeruginosa* and *S. aureus* were identified to the maximum delicate strain compared with standard streptomycin drug and the zone will be developed while increasing the sample. Observed and *E. coli*is also a susceptible strain compared with standard streptomycin drug but the zone is not developed in (500 µg and 1000 µg) while increasing the sample.

Anti-inflammatory activity

The anti-inflammatory activity of Nickel Oxide (NiO) nanoparticles was assessed using a membrane-stabilization assay based on red blood cell (RBC) protection (Table4). In this procedure, blood (1 mL) was combined with an equal volume of Alsever's solution (comprising 2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl) and centrifuged at 3,000 rpm for 5 min. After discarding the supernatant, the red blood cells (RBCs) were isolated, washed with isosaline (0.85% NaCl), and centrifuged again at 3,000 rpm for 5 min. A 1 mL aliquot of RBCs was then diluted with 9 mL of isosaline to prepare a haemoglobin-rich RBC (HRBC) suspension. Different concentrations of the NiO sample (100 µg, 200 µg, 300 µg, 400 µg, and 500 µg) were set up in test tubes, each containing 1 mL of phosphate buffer, 2 mL of hypo saline (0.36% NaCl), and 0.5 mL of the HRBC suspension. The mixtures were incubated at 37ºC for 30 min and then centrifuged at 3,000 rpm for 20 min, after which the absorbance of the supernatant was measured at 560 nm. Diclofenac (1 mg/mL) was used as a standard, and control was prepared without the sample[39]. The

Table 3 — The table shows the bacterial activity of NiO NPs

Sample/Microorganism	Zone of Inhibition in mm					
	1000 µg	500 µg	250 µg	125 µg	DMSO	1000 µg
S. aureus	13	12	11	10	00	26
B. subtilis	13	12	10	-	00	23
E. coli	11	11	10	-	00	18
P. aeruginosa	12	10	-	-	00	17

Table 4 — Anti-inflammatory activity membrane-stabilization assay based on red blood cells (RBC)

Sample absorptions	100 µg	200 µg	300 µg	400 µg	500 µg
Diclofenac	0.36	0.27	0.21	0.17	0.12
% inhibition	12.1	34.1	48.7	58.5	70.3
Std Blank =0.41					
Nickel oxide sample	0.36	0.35	0.34	0.33	0.32
% inhibition	2.7	5.4	8.1	10.8	13.5
Bank = 0.37					

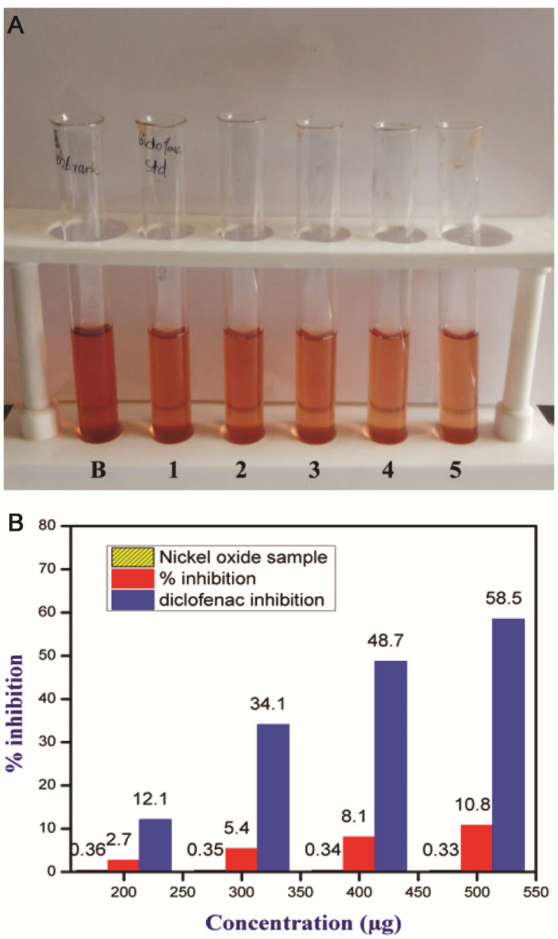

Fig. 9 — (A) Anti-inflammatory Activity- Membrane assay method; and (B) Anti-inflammatory activity

percentage of HRBC protection was calculated as follows:

Percentage of Protection (%) = 100 - [(OD of sample/OD of Control) × 100]

Compared to the standard diclofenac, which exhibited a maximum inhibition of 70.3% at 500 µg and a minimum of 12.1% at 100 µg, the NiO nanoparticles showed lower inhibition rates, with a maximum inhibition of 13.5% at 500 µg and a minimum of 2.7% at 100 µg. Despite this modest anti-inflammatory effect, NiO nanoparticles hold promise as a safer option for long-term use due to their biocompatibility and low toxicity. This study introduced the concept given by Rudolf Virchow, who linked inflammation to cancer risk, indicating that materials like NiO nanoparticles may have the potential for reducing inflammation-associated risks in a safer way[38]. (Fig. 9A &B) shows the anti-inflammatory activity membrane-stabilization assay based on red blood cells (RBC).

Conclusion

The green production of Nickel Oxide nanoparticles utilizing *Vitex negundo* leaf extract was effectively completed. The XRD pattern indicates a face-centered cubic structure, with an increase in average crystallite size corresponding to higher annealing temperatures. Optical studies, performed using UV characterization, revealed a bandgap energy of 3.38 eV. FT-IR characterization identified the functional groups present, while morphological studies via SEM characterization showed that the particles are spherical with some agglomeration. The elemental ratio was determined using EDX characterization. The application of these Nickel Oxide nanoparticles demonstrated significant antibacterial activity against *B. subtilis*, *S. aureus* and *P. aeruginosa*, while exhibiting lower effectiveness against *E. coli*. However, the anti-inflammatory results indicated weak activity, which may be attributed to the non-toxic nature of Nickel Oxide nanoparticles. Looking ahead, future work will explore the addition of suitable dopants to enhance the anti-inflammatory activity, potentially increasing the therapeutic effectiveness of these nanoparticles.

Conflict of interest

All authors declare no conflict of interest.

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