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Synthesis of Nickel Oxide Nanoparticles (NiO-NPs) from *Aeschynomene Indica* and its Antibacterial activity

M. John Merlin[a,b], S.Joseph Selvaraj[b], A. Peter Pascal Regis[b], G. Hema Sindhuja[c]
[a]PG Department of Chemistry, Ananda College, Devakottai – 630303, Tamil Nadu, India
[b]PG & Research Department of Chemistry, St. Joseph's College,Tiruchirappalli- 620002, Tamil Nadu, India
[c] PG & Research Department of Chemistry, Jamal Mohamed College, Tiruchirappalli - 620020, Tamil Nadu. India
Corresponding Authors: A. Peter Pascal Regis
Email: drpascalregis@gmail.com

ABSTRACT
Nanomaterials have gained interest in multisector due to their size and specific optical properties. Metal oxide nanoparticles found its greater height in nanotechnology with various applications. The current study reports the synthesis of Nickel oxide nanoparticles using Aeschynomene Indica leaf extracts. Synthesised NiO-NPs were characterized using various techniques like UV-visible spectroscopy (UV-vis), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), Energy dispersive spectroscopy (EDS), Scanning electron microscopy (SEM) etc. The size of the NPs was about 157 nm which also shows a high crystalline nature. FTIR analysis revealed that the strongest bond corresponds to the stretching vibration mode of NiO nanoparticles. Nickel oxide nanoparticles (NiONPs) show good antibacterial activities against Enterococcus faecalis bacteria.
Keywords: *Metal oxide Nanoparticles, crystalline, gram positive bacteria*

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INTRODUCTION
Nanotechnology is one of the most important and fast-rising areas in the field of science and engineering, involving the combination of familiarity from chemistry, biology, materials science and other related branches. Nanoparticles (NPs) have a very high surface-to-volume ratio because of their exact small size. Based on their shape, nanoparticles are known as Nano flowers, nanocubes, nanowires and nanotubes etc. [1,2,3] NPs have a wide range of applications in altered profitable areas such as cosmetics, food, drug delivery, agriculture, cancer finding, cancer therapy and photocatalysts.[4] The extent of NPs applications is due to unique properties such as optical, chemical, magnetic, mechanical electronic etc. Metallic nanoparticles expressively differ in many properties from that of their bulk materials such as size, shape, electrical, surface effect and magnetic properties. [5, 6] NiO-NPs have attracted the attention of the scientific community due to their multifunctional and tunable nature. Nickel oxide (NiO) is a p-type wide band gap (3.6 to 4.9 eV) semiconductor with high chemical solidity and electron transfer ability [7]. NiO nanoparticles possess characteristic properties such as large surface area-to-volume ratios, high photo absorption, high dispersion rates, low porosity and low heat capacities [8]. These unique properties of NiO nanoparticles make feasible and cost-effective surfaces proper for different applications like hydrogen storage photocatalytic degradation of organic dyes, pollutants from waste water and antimicrobial activity [9,10, 11]. So far, numerous synthesis methods like electrochemical reduction [10], chemical reduction, [12], sol-gel [13] has been described to synthesize NiO nanoparticles. The nanoparticles produced by plant extracts are more stable and biocompatible in contrast with those produced by physical and chemical methods [14]. However, these physicochemical methods have numerous injurious side effects which limit their wide range of biological applications. [15] Chemical synthesis most regularly generates toxic chemical waste leading to environmental toxicity and non-biodegradable products while that of physical methods needs enormous energy input. [16, 17].
In plant referred synthesis of nanoparticles, extract of different parts of a plant is used as reducing agent and stabilizing agents [18, 19]. Recently, NiO nanoparticles have been synthesized using microorganisms, enzymes and plant extracts. [20] The plant extract can act as a strong reducing, stabilizing and capping

agent and has strained the care of the scientific community due to its simple, fast, cost-effective and eco-friendly nature. [22, 21] The determination of this study was to synthesize NiO-NPs using leaf extract of *Aeschynomene Indica*. The *Aeschynomene Indica* is an amusing source of alkaloids, flavonoids and anthraquinones compounds and can be used as a natural precursor in the formation of biogenic nanoparticles. The plant is comprised of emodin, kaempferol, physcion, herbacetin, rhamnocitrin, quercetin, and maesopsin which induced the strong reducing/stabilizing and capping agents. [23, 24] The ethnobotanical study and fiction review have reported the medicinal values of *Aeschynomene Indica* and have shown strong therapeutic potentials such as antimicrobial, laxative, antioxidant, emetic and purgative mostly used in the treatment of spleen infection parasitic infections and in leg swelling. [25, 26, 27] NiO nanoparticles possess biomedicinal applications for cancer cell treatment.[28] Because of their anti-inflammatory property, NiO-NPs can also be used in biomedicines. [29]

MATERIAL AND METHODS

All the chemicals and reagents were of Analytical grade. Nickel Chloride ($NiCl_2$), were purchased from Merck and ethanol from SRL were used for leaf extraction process. Double distilled water has been used for the entire study

Preparation of *Aeschynomene indica* Leaf extract)

The leaves of *Aeschynomene indica* were collected from Devakottai, Tamilnadu, India. The collected plant leaves were washed with distilled water several times and dried under shade. Then the dried leaves are crushed and converted into fine powder which was used for extraction process. The ethanolic leaf extract process is given briefly [30].100g of leaf powder was mixed with 100 mL of ethanol in a 250 ml beaker. The mixed solution was kept for 5 days and the solution was filtered using Whatman filter paper. The filtrate was named as *Aeschynomene indica* extract which was used for synthesis of NiO-NPs.

Synthesis of Nickel Oxide Nanoparticles

About 2 gm of $NiCl_2.2H_2O$ was taken and made into a solution and stirred for 2 hr with magnetic stirring with 400 rpm speed and maintained 80^0C throughout the experiment. The ethanolic *Aeschynomene indica* extract was added slowly into the Nickel solution until the precipitate is obtained. The Precipitate was dried and converted into fine powder which was then transferred into a china crucible and kept in a muffle furnace for incineration for about 6 hours at 450^oC. The metal oxide salt thus formed was ground well to make a NiO-NPs. [31],

Characterization

Characterization of Nickel oxide nanoparticles was carried out using various techniques. Fourier transforms infrared spectroscopy (FT-IR PerkinElmer) with a range from 4000 cm^{-1} to 400 cm^{-1}. The absorbance value was recorded using UV–visible spectrophotometer with a range of 200-800 nm. The surface morphology and shape of the nanoparticles were determined by Scanning Electron microscopy (SEM). Nanoparticle composition was analysed using Energy Dispersive X-Ray Analysis (EDAX). X-ray diffractometer, with Cu Kα radiation (λ = 0.1546 nm) and diffraction angle between 10 - 90° was used to predict the crystalline nature of nanoparticles.

Antibacterial Activity

The antibacterial activity of synthesized NiO-NPs were tested against ***Enterococcus faecalis, Pseudomonasa aeruginosa and Streptococcus oralis*** bacteria by Muller Hinton agar well diffusion method. New bacterial strains were cultured by nutrient broth as cultured medium. In brief, an adequate amount of 2.8-3.0 g of nutrient agar medium was dissolved in DI-water. The dissolved agar medium was autoclaved at 121^oC for 15 minutes with pressure of 15 lbs. The agar medium was mixed well under molten condition and feed onto 100mm petri plates. The warm agar medium was mixed with bacterial inoculums (100 µL). Petri plates containing 20 ml of nutrient-agar medium was allowed to solidify for 24h and adjusted to 0.5 OD value. At this stage Bio-synthesized NiO-NPs were placed onto the disc at four distinct concentrations (50, 100, 250 and 500 µg/ml) using sterilized forceps. A well-known antibiotic gentamicin was fixed as positive control (PC) through-out this study. The petri plates were allowed to incubate for 24h at 37^oC and the experiment were evaluated on measuring the zone inhibitory diameter in millimetres (mm). All the experiments were assessed in three separate plates and summed to retrieve concordant data.

RESULTS AND DISCUSSION

Analysis of UV-Visible absorption spectra

The UV-Visible spectrum of synthesized NiO-NPs is shown in Figure 1. which indicates that the peak is observed at 392.55.nm (1.3114AU), 725.45nm (1.1315AU), and 906.10nm (1.0903AU) suggests the reduction of Nickel and the formation of Nickel oxide nanoparticles. NiO nanoparticles are considered stable, and this fact can be the result of a symmetrical polarity structure which depends on the weak interaction of Van der Waals forces within the particle regime.

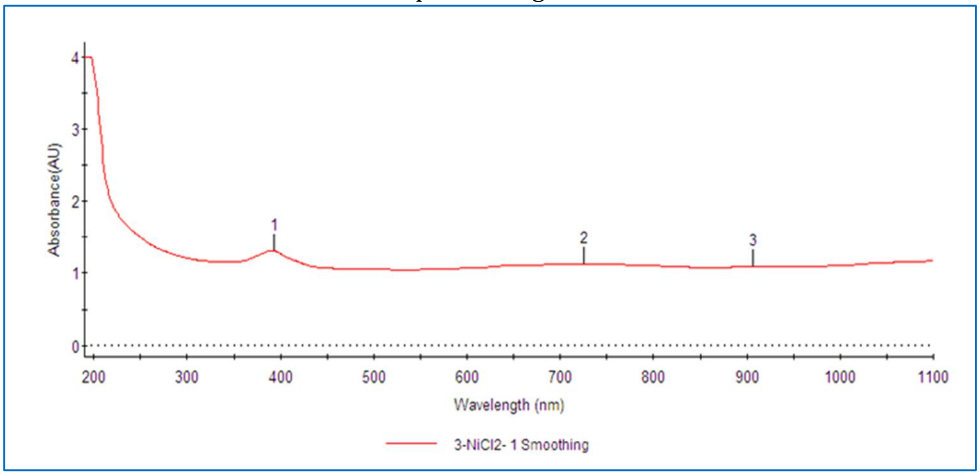

Figure 1. UV-Visible spectra of NiO nanoparticles (NiO-NPs)

Figure 2. shows the FTIR spectrum of NiO-NPs where peaks were observed at 3431cm^{-1}, 1614 cm^{-1}, 1401 cm^{-1} and 1113 cm^{-1}. The strong bond corresponding to Ni-O stretching vibration mode of NiO nanoparticles is observed. The bonds are characteristic of a hydroxyl group (OH) , which may rise due to the adsorption of water molecules onto to NiO surface while preparing the sample and exposure to atmospheric air.

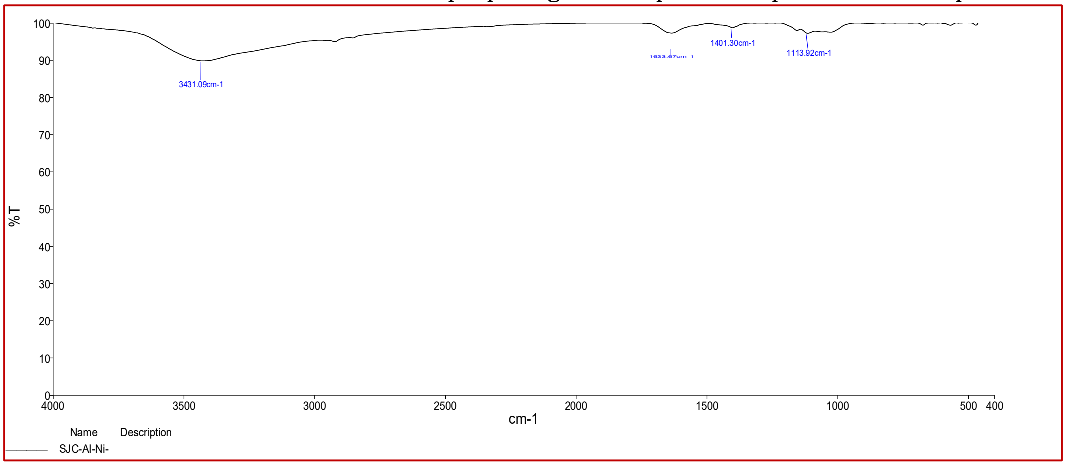

Figure 2. FT-IR spectra of NiO nanoparticles (NiO-NPs)

X-Ray Diffraction (XRD).

Figure 3. Shows the XRD pattern of synthesized Nickel oxide nanoparticles (NiO-NPs) Powder X-ray diffraction was used to confirm diffraction angles and the sample showed a high level of crystallinity. However, there are a few peaks at 2θ values of 31.72, 37.22, 43.25, 62.83, 75.36, and 79.32 which corresponds to the Ni planes (210), (311), (400), and (511). The d-spacing values of Ni nanoparticles are (d_{hkl}-2.82, 2.41, 2.08, 1.47 and 1.26). The diffraction values are 0.11, 0.09, 0.07, 0.14, and 0.19 in full-width half maximum (FWHM). The confirmation of the successful synthesis of Nickel oxide nanoparticles was done by powder X-ray diffraction (XRD).

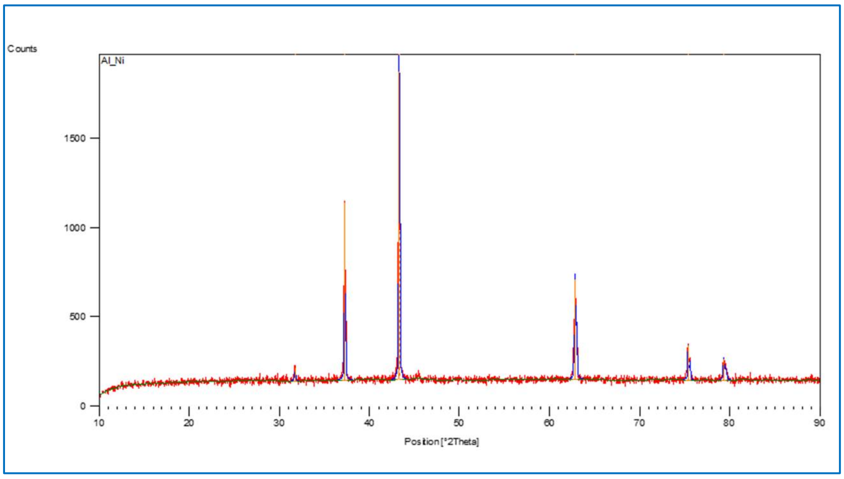

Figure 3. X-Ray Diffraction of NiO nanoparticles (NiO-Nps)

SEM and EDAX analysis

Figures 4 (a) and (b) show the SEM images of NiO nanoparticles with different magnifications, which clearly exhibit well-uniform particles with size distribution in the range of 157 nm. The surface morphology of the NiO nanoparticles has a smooth surface, which facilitates better contact with the bacterial cell wall and hence increases the bacterial killing ability of NPs. The elemental composition of the synthesized NiO-NPs was evaluated from EDAX analysis which is shown in Figure 4 c. The major peaks indicate the Ni and O of the synthesized NPs. However, some minor peaks of Carbon, Calcium, Potassium, Chlorine and Silicon are also present which are attributed to the plant extract used. The elemental composition of the nanoparticles shows 26 wt% Nickel and 68 wt% Oxygen corresponding to Nickel oxide (NiO). The compositional data from the EDAX analysis agree well with theoretically calculated values, indicating a good compositional homogeneity across the nanoparticles.

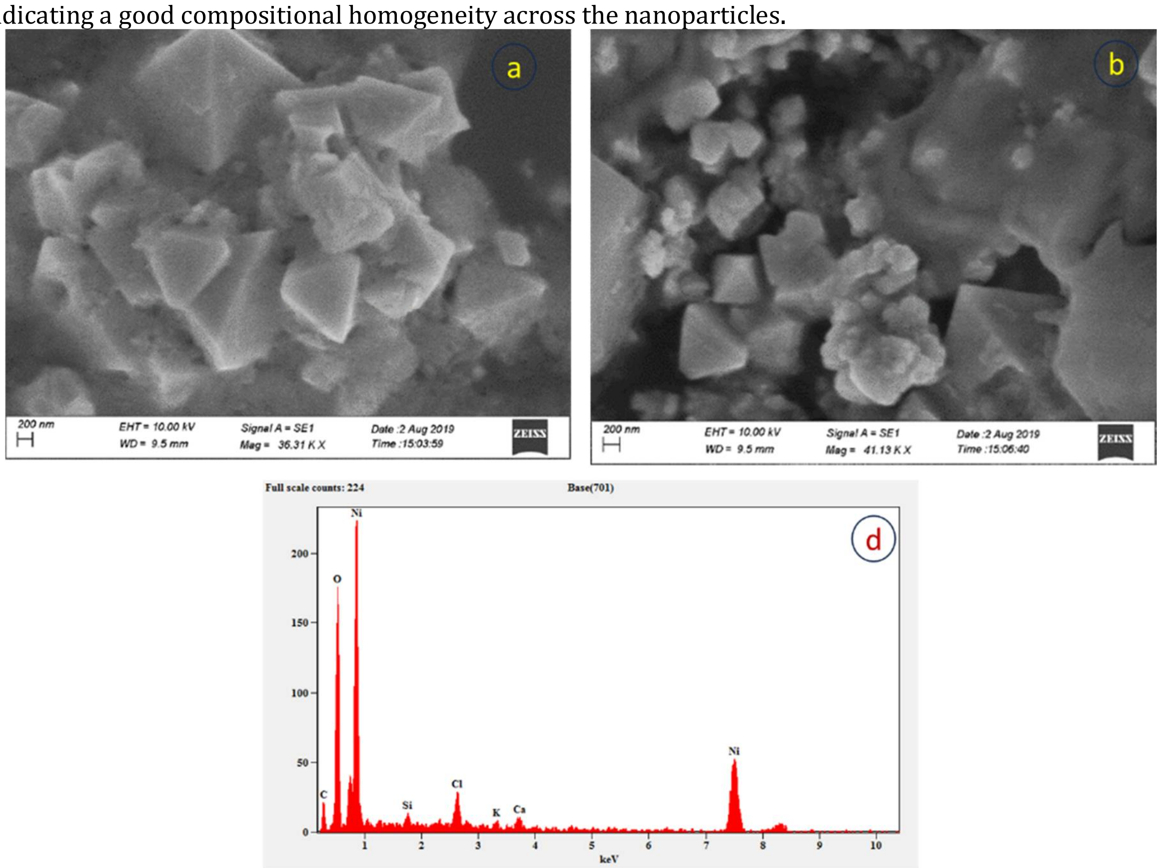

Figure 4. SEM micrographs of Nickel oxide nanoparticles with different Magnification (a) 36.31Kx (b) 41.13Kx

Antibacterial assay

The antibacterial activity was performed by well diffusion methods. To measure the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA). compound highly active against Enterococcus faecalis bacteria when compared to Streptococcus oralis and *Pseudomonas aeruginosa.*

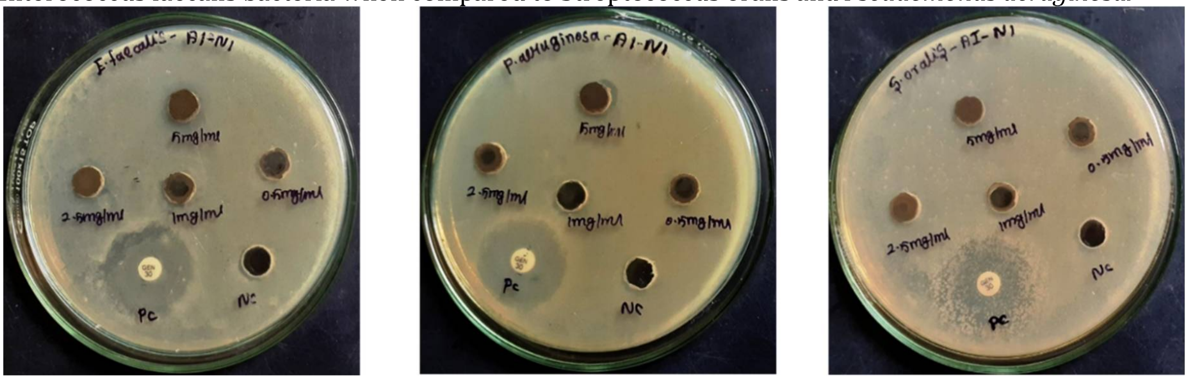

Figure 5. Antibacterial study of NiO-NPs against *Enterococcus faecalis, Pseudomonasa aeruginosa and Streptococcus oralis*

S.NO	Name of the test sample	Name of the test organism	Zone of inhibition (mm)				
			500 µg/ml	250µg/ml	100 µg/ml	50µg/ml	AB
1	Aeschynomene Indica-Nickel Oxide Nanoparticles	*Enterococcus faecalis*	6.5±0.7	4.25±0.35	4.2±0.28	0	15.5±0.7
2		*Pseudomonasa Aeruginosa*	5.5±0.7	0	0	0	16.5±0.7
3		*Streptococcus oralis*	0	0	0	0	16.5±0.7

Table 1. SD± Means of zone of inhibition obtained by Aeschynomene Indica –Antibacterial Nickel Oxide Nanoparticles against *Streptococcus oralis, Pseudomonasa aeruginosa and Enterococcus faecalis*

CONCLUSION

The current study described the one-pot synthesis of NiO-NPs using leaf extract of *Aeschynomene Indica* as both reducing and stabilizing agent. Nickel Oxide nanoparticle size was about 157 nm. The surface morphology indicates a smooth surface which plays a vital role in antimicrobial activity. Antibacterial activities of synthesized Nickel oxide nanoparticles were analysed against *Enterococcus faecalis, Pseudomonasa aeruginosa and Streptococcus oralis* bacteria and it was found that increasing the concentration of Nickel oxide nanoparticles, antibacterial activity was increased against *Enterococcus faecalis* bacteria. Since the synthesized nanoparticles possess biocompatibility and antibacterial activity and thus can be used in the field of biomedicine.

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