ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESISED NICKELOXIDE NANOPARTICLES ASSISTED BY *TERMINALIA CHEBULA*

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Abstract:

In this study, ecofriendly and simple green route was employed for the synthesis of nickel oxide nanoparticles. The NiO nanoparticles were prepared using Nickel chloride and dried fruit extract of *Terminalia chebula*. The UV-Vis analysis shows blue shifted peak at 317 nm depicting the smaller size of the synthesized nanoparticles as compared to bulk NiO. FTIR spectrum of the NiO nanoparticles shows the presence of vibration modes of Ni-O bonds and that of the phytoconstituents in the plant extract confirming the role of plant extract as the capping agent. The XRD pattern clearly indicates the formation of face centered cubic (FCC) crystal lattice of NiO nanoparticles and the average size of NiO nanoparticles was calculated using scherrer's formula as 53.43 nm. The AFM study of NiO nanoparticles pictured the irregular rough surface of the nanoparticles. The antibacterial activity of the green synthesised NiO nanoparticles against various gram positive and gram negative bacteria revealed that they are highly active against bacteria in polar solvents.

Keywords: Green synthesis, Nickel Oxide Nanoparticles, *Terminalia chebula,* Antibacterial activity.

1. Introduction:

Recently research on transition metaloxide nanoparticles has led to the development of various devices having enhanced properties [1]. The transition metal cations have incomplete d-orbitals which play a major role in imparting unique characteristics. Due to the development of resistant strains, resistance of bacteria to bactericides and antibiotics has increased. Some antimicrobial agents are extremely toxic and there is vital need and much interest in finding ways to formulate new type of safe and cost effective biocidal material [2].

NiO is considered as p-type semiconductor. They are chemically stable and have superconducting, magnetic, electrocatalytic, electron transfer and antibacterial properties [3]. They are prepared by various chemical and physical techniques while bionanotechnology method uses plants, bacteria, virus, fungi, yeast and animal sources for the synthesis of NiO nanoparticles [4]. The chemical constituents of *Terminalia Chebula* like chebulinic acid, tannin, gallic acid and ascorbic acids are the key factor for the efficient action of the species. 32-34% of the total phytoconstituents are hydrolysable tannins which can effectively act as a capping agent in the synthesis of NiO nanoparticles [5]. Herein, this work reports the antibacterial activity of NiO nanoparticles synthesized using dried fruit extract of Terminalia chebula.

2. Materials and Methods

2.1. Materials

AR grade Nickel chloride ($NiCl_2$) was purchased from Hi Media Chemicals, Mumbai. Deionised water was used throughout the experiment. The fruits of *Terminalia chebula* were procured from the market of Thoothukudi district, Tamilnadu. It was washed well with distilled water, shade dried, powdered and sieved. used for extract preparation. About 10g of the powder was added to 100ml of double distilled water, heated for 30 min and filtered through Whattman No.41 filter paper. The extract was stored in refrigerator for the synthesis of nickel oxide nanoparticles.

2.1. Synthesis of Nickel oxide nanoparticles

Inorder to prepare nickel oxide nanoparticles, 10 mL of 0.1M nickel chloride solution was made upto 100 mL. To this solution, 10mL of *Terminalia chebula* fruit extract was added and stirred for 30 min.The stirred solution was heated at 80ºC for 2 h. The brown residue thus obtained was collected in a clean and dry silica crucible. It was heated to 400ºC for 2h in a muffle furnace. The black colored Nickel oxide nanoparticles thus obtained was collected, preserved and used for further characterizations and applications.

2.2. Antibacterial assay

A loopful of the test organism was transferred to already sterilized 10 ml Nutrient agar and incubated overnight at 37^0C. *Aspergillusniger* was cultured as a slant culture in an acidified PDA (Potato Dextrose Agar) media. 25 ml of sterilized Muller-Hinton Agar (MHA) (Hi Media, Mumbai, India) was poured in petriplates and allowed to solidify at room temperature on which the test organisms were inoculated. The antimicrobial activity was measured by Disc Diffusion method. The plates are seeded with 15µL of the sample and incubated at 37^0C for 24 hours. The antibacterial activity was recorded as the mean diameter of the resulting inhibition zone of growth measured in millimetres. From the results, the Active Index (AI) and Proportion Index (PI) were calculated using the formulae (1) and (2).

$$\text{Active Index (AI)} = \frac{\text{Inhibition zone of the test sample}}{\text{Inhibition zone of the standard}} \quad (1)$$

$$\text{Proportion Index (PI)} = \frac{\text{Number of positive results obtained for individual extract}}{\text{Total number of tests carried out for each extract}} \quad (2)$$

3. Result and Discussions

The UV-Vis spectrum of NiO nanoparticles (Fig.1.a) show peak at 317 nm is due to charge transfer transitions from valence band to the conduction band of NiO nanoparticles. It is blue shifted when compared with the absorption wavelength (331 nm) of bulk NiO particles. The additional peak at 258 nm is due to the π - π^* transitions of the chemical constituents present in the dried fruit extract which acts as a capping agent.

Fig.1.b shows the FTIR spectrum of the NiO nanoparticles. The bands at 442 cm^{-1} and 668 cm^{-1} can be attributed to Ni-O stretching and Ni-O-H stretching. The bands located at 1109 cm^{-1}, 1384 cm^{-1} and 1464 cm^{-1} can be assigned to C-O stretching of carboxylic acid, –OH bending of carboxylic and phenolic groups and -C=O stretching respectively. The peaks at 1585, 2360 and 2923 cm^{-1} can be attributed to aromatic –C=C- stretching, O-C=O stretching, aromatic –CH stretching modes respectively. The broad peak at 3438 cm^{-1} corresponds to the OH stretching vibrations of phenolic compounds like gallic acid etc., present in the fruit extract.

The XRD peaks (Fig.1.c) at 37.44º, 43.46º, 63.02º, 75.53º, 79.49º correspond to the planes 111, 200, 220, 311, 222 (JCPDS, No.04-0835) indicating FCC lattice structure. The average size of synthesized Nickel oxide nanoparticles was calculated to be 53.43 nm using the scherrer's formula $D = k\lambda / \beta\cos\theta$. The obtained XRD pattern clearly indicated the formation of nano NiO and showed no other diffraction peaks, thus confirming the purity of NiO phase.

Fig.1.d. shows the AFM spectral image of NiO nanoparticles synthesized using *terminalia chebula* fruit extract with a scanning area of 9.842 pm^2 between 0 m X 3.13 μm and 0 m Y 3.13 μm. The surface of the nanoparticles were found to have irregular and rough nature.

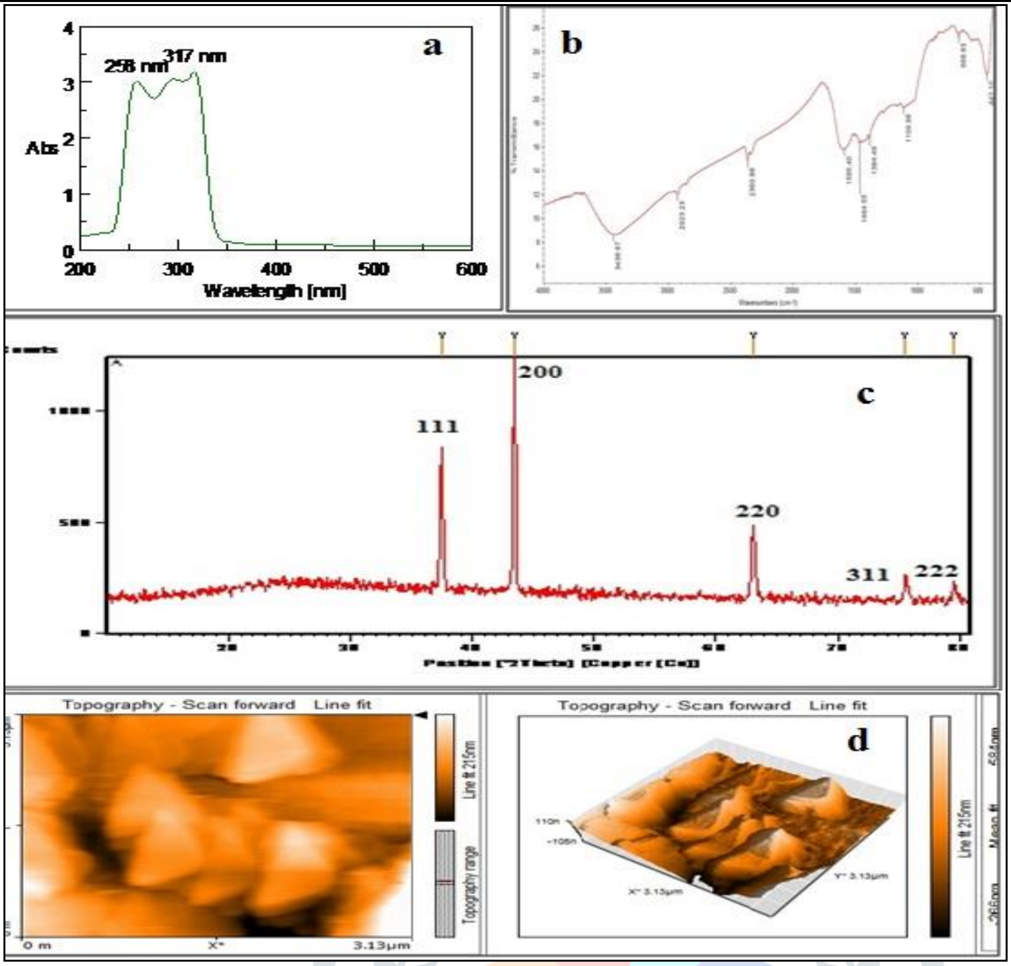

Fig.1. a - Uv-vis Spectrum , b- FTIR spectrum, c-XRD pattern, d- AFM spectrum of NiO Nanoparticles synthesized using *Terminalia chebula* fruit extract.

4. Antibacterial activity

The antibacterial study of the green synthesized NiO nanoparticles in different solvents were established against pathogenic gram positive (*Bacillus Cereus, Bacillus subtilis Staphylococcus aureus)* and gram negative (*Escherichia coli, Klebsiella pneumonia Salmonella typhi)* bacteria. The antibacterial activity in terms of inhibition zone significantly varies with different test bacteria in different solvents (Table1).

The results suggested that the NiO nanoparticles suspended in water solvent is found to be more active than the standard, against all bacteria under study. While NiO nanoparticles in ethanol is found to be more active than the standard, against gram negative bacteria. The NiO nanparticles in benzene was found to be inactive against all the pathogenic bacteria under study except gram positive *Bacillus subtilis* (AI=0.92). Thus it can be concluded that the synthesized NiO nanoparticles in polar solvents are highly active while the same in nonpolar solvents are rendered inactive against the pathogenic bacteria under study.

Table.1. Zones of inhibition of NiO nanoparticle against various test Bacteria

Name of the organism	Zone of Inhibition (mm)										
	Petroleum ether (40^0-60^0C)		Benzene		Chloroform		Ethanol		Water		Stds$^+$
	DIZ*	AI$^\#$	DIZ*	AI$^\#$	DIZ*	AI$^\#$	DIZ*	AI$^\#$	DIZ*	AI$^\#$	
Bacillus Cereus	6	0.50	-	0	-	0	5	0.42	9	0.80	7
Bacillus subtilis	-	0	9	0.92	-	0	-	0	10	1.01	8
Staphylococcus aureus	-	0	-	0	-	0	20	1.17	14	0.82	12
Escherichia coli	-	0	-	0	10	0.62	19	1.12	16	0.94	11
Klebsiella pneumonia	12	1.00	-	0	-	0	22	1.15	18	1.15	16
Salmonella typhi	-	0	-	0	6	0.38	19	1.19	22	1.38	14
*DIZ - Diameter of zone inhibition; $^\#$AI - Active Index; - No inhibitory effect; Stds$^+$ - standards											

5. Conclusion

The Nickel oxide nanoparticles were successfully synthesized using aqueous dried fruit extract of *Terminalia chebula* using Nickel chloride as a precursor. The blue shifted UV-Vis absorption peak at 317 nm confirmed the nano-size of the synthesized NiO nanoparticles. The FTIR interferrogram reveals the presence of phytoconstituents like carboxylic acids and polyphenols which acts as the surface active molecules, stabilizing the nanoparticles by interacting with NiO lattice surface. The presence of these functional groups of biomolecules in the fruit extracts of *terminalia chebula,* responsible for stabilization of Nickel oxide nanoparticles was confirmed by FTIR analysis. XRD data clearly showed the crystalline nature of Nickel oxide nanoparticles. The size of the NiO nanoaprticles having FCC lattice structure was calculated from the x-ray diffractogram using debye scherrer formula and was found to be 53.43 nm. AFM study revealed the topography of the synthesized NiO nanoparticles. The synthesized Nickel oxide nanoparticles in water and ethanol solvent showed enhanced antimicrobial activity against various gram positive and gram negative bacteria. Thus, these biosynthesized NiO nanoparticles can be used as effective growth inhibitors of various microorganisms, making them useful in diverse medical devices and antimicrobial control systems.

6. References

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