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Antibacterial Activity of Nickel Oxide nanoparticles Synthesized from Tulsi (*Ocimumtenuiflorum*) Leaves Extract

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ABSTRACT

Synthesis of nickel oxide nanoparticles using biological entities is cost effective and eco-friendly approach. In current study, nickel oxide nanoparticles (NiO) synthesized by utilizing tulsi (Ocimum tenuiflorum) leaves extract which reduces nickel chloride precursor into its nano form. The structural properties of nanoparticles were confirmed by UV- visible spectroscopy, Fourier transform infrared spectroscopy (FTIR) and X- ray diffraction technique (XRD). The NiO nanoparticles exhibited characteristic peak at 379 nm with band gap 3.27 eV while FTIR spectroscopy analyzes different functional groups attached to it. In X-ray diffraction analysis NiO nanoparticles revealed sharp and highest peak at (220) with 2 θ value 52.59 and also exhibited single crystalline structure having tetragonal cubic phase with 3.37 nm size. The biologically synthesized nickel oxide nanoparticles from Ocimum tenuiflorum leaves extract showed antibacterial activity against Gram positive Staphylococcus aureus and Gram negative Escherichia coli.Thus,NiO nanoparticles can be utilized as potential antibacterial agent against range of micro-organisms.The synthesis of NiO nanoparticles using leaves extract is more appropriate strategy for the effective synthesis of nanoparticles.

KEYWORDS

Ocimum tenuiflorum, Nickel oxide nanoparticles, Staphylococcus aureus, Escherichia coli, Antibacterial agent.

1. INTRODUCTION

Nanoscience and Nanotechnology is the innovative branch of science that deals with production of nanoparticles with improved properties which are contrast from subsequent solid-state material and its applications. The size and properties

alterations are due to quantum size, surface to volume ratio and macroscopic tunnelling effect. The various physical and chemical techniques were employed for synthesis of metal oxide nanoparticles. But these methods are costly, time consuming and may cause some environmental effects due to utilization of harmful chemicals. Therefore, biological method for nanoparticles synthesis was developed to minimize impacts of harmful chemicals. Synthesis of nanoparticles using biological entities iseco-friendly, cost effective, minimum use of chemicals and requires less time for synthesis [1].

Amongst the all metals, magnetic transition metal dependent nanoparticles of Nickel, Copper and Iron was synthesized due to its superior magnetic properties and various applications. The crucial feature of Nanobiotechnology is the combination of biological principles with physical and chemical approach so as to synthesize nano sized particles with distinctive functions. Several procedures are performed to control the size and shape of nanoparticles [2]. The various biological things were employed for synthesis of nanoparticles but utilization of plant extracts specifically leaves showed significant formation of nanoparticles. The several secondary metabolites like terpenoids, flavonoids, phenols and alkaloids were present in leaves extract. These metabolites act as reducing agent which reduces bulk precursor into its nanoparticle form. The solvents with varying degree of polarity also effects on the synthesis of metallic nanoparticles [3]. There are certain basic requirements in biological synthesis; it includes i) choice of proper solvent, ii) an eco-friendly reducing agent and iii) non-toxic stabilizing agent for nanoparticles. Therefore, by maintaining suitable conditions biological method produces nanoparticles with controlled morphology without generation of harmful by-products. The materials synthesized by eco-friendly approach possess wide applications in biomedical and pharmaceutical industries [4]. The advantages behind the utilization of plant extract mediated metal nanoparticle synthesis is that, they are easily available, safe, non-toxic and contain large variety of phytochemicals which acts as effective reducing and capping agent. Thus, due to these benefits plant extracts easily converts bulk precursor into its nano form than microbial and fungi mediated nanoparticles synthesis [5].

Ocimum tenuiflorum, a small medicinal herb belongs to the family Lamiaceae commonly known as Holy Basil or Tulsi. The phytochemical constituents of tulsi consist of oleanolic acid, ursolic aid, rosmarinic acid, eugenol etc. while the essential oils are β-elemene, β-caryophyllene and germacrene. There are two nano forms of nickel that are nickel metal and nickel oxide. These two classes exhibited specific properties like magnetic, biocompatibility, catalytic activity, antimicrobial potential and sorption nature. Also, this nano sized nickel oxide nanoparticles (NiO) with band gap 3.6-4.9 eV behaves as semiconductor with high chemical stability and electron transfer efficiency. Hence, these NiO nanoparticles have wide applications in diverse

fields like electronics, energy devices, nanomedicines, sensors, waste water treatment and in the various organic synthesis such as reduction, hydrogenation, alkylation etc. [6]. In recent decades, plant extracts, micro-organisms, fungi and some enzymes were exploited for the synthesis of NiO nanoparticles. Thus, it results in development of green protocol approach.Among, all the biological things, plant extract attracts more attention because it acts as an effective capping agent and also helps to control size morphology of nanoparticles to prevent its agglomeration [7].

In current study, the synthesis of nickel oxide nanoparticles using *Ocimum tenuiflorum* leaves extract was carried out by using nickel chloride as precursor while ethanol and water used as solvent. The synthesized nanoparticles were characterized by UV-visible spectroscopy; Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). The antibacterial activity of prepared nanoparticles were studied against Gram positive and Gram negative micro-organisms.

2. EXPERIMENTAL METHODOLOGY

2.1. Preparation of Ocimum tenuiflorum leaves extract and precursor

Fresh leaves of *Ocimum tenuiflorum (tulsi)* were collected from botanical garden of Y.C.I.S. Satara, Maharashtra India. The leaves were washed with distilled water (D/W) to remove debris on it. These leaves were cut into fine pieces and crushed with minimum amount of D/W. 25 gm of crushed leaves were stirred along with 100ml of D/W and ethanol in 1:1 proportion at 80°C for 2 hr. After stirring, resultant mixture was filtered through Whatmann filter paper and filtrate was used for synthesis of NiO nanoparticles. Pure 0.1mM nickel chloride (HiMedia,India) was dissolved in 100 ml of D/W and utilized as precursor for nanoparticle synthesis.

2.2. Synthesis of Nickel Oxide nanoparticles

The prepared leaves extract was mixed with 0.1 mM nickel chloride in 1:3 proportions with drop wise addition and stirring at 80°C for 3 hr on magnetic stirrer. The resulting solution was annealed in furnace at 200°C for 2 hr. After annealing, black brown colored powder was obtained. Further, it was washed with ethanol to remove any other impurities. The purified powder was employed for further characterization and antibacterial studies.

2.3. Antibacterial activity

The bacterial strains were collected from Department of Biochemistry, SUK. The antibacterial activity of synthesized NiO nanoparticles was studied against Gram positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* by using agar well diffusion method. The nutrient agar plates were prepared by traditional method and 100µl of *S.aureus* and *E.coli* suspension was spread on plate with sterilized spreader. Well, were prepared on each plate with the help of a sterilized borer. Add 50 µland 100 µl of synthesized nanoparticles into 2 wells while 50 µl of leaves

extract in another well as control. Plates were kept for incubation at 37°C for 24 hr. Zone of inhibition was observed on next day to determine the antibacterial activity. The zone was measured to determine the effectiveness of nickel oxide nanoparticles against these pathogens.

3. RESULT AND DISCUSSION

3.1. UV-Visible spectroscopy

The colour variation in solution is determined by UV–visible spectroscopy which is the basic characterization technique to study nanoparticles. The UV–visible spectra of synthesized NiO nanoparticles were noted at room temperature between 200 and 800 nm against D/W as a reference. The **Figure-1** indicates the UV-Visible spectrum of synthesized NiO nanoparticles from *tulsi* leaves extract. Due to surface plasmon resonance, NiO nanoparticles exhibited characteristics peak at 379 nm with band gap (Eg) 3.27 eV. The UV absorption also depends on size and shape of nanoparticles [8].

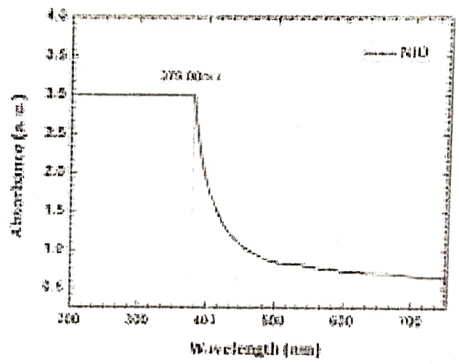

Figure-1. UV Visible spectrum of NiO nanoparticles.

3.2. Fourier transforms infrared spectroscopy (FTIR)

The FTIR spectroscopyanalysis of synthesized NiO nanoparticles was carried out between 500 and 4000 cm-1. The different functional groups attached to NiO nanoparticles surface were analysed by FTIR spectroscopy. The **Figure-2** represents the FTIR spectrum of prepared NiO nanoparticles. The small peak at 580.28 cm-1 corresponds to metal-oxygen stretching frequencies are related to Ni-O [8]. The small absorption band at 2920.68 cm-1 is due to the extending vibration mode of CO2, which occurred due to presence of aerial CO2 or CO2 inside the grains of the nanoparticles [9]. The broad absorption peak observed nearly at 3397.44 cm^{-1}related to OH functional groups [10]. The band between the region of 1000–1500 $cm^{-1}$are symmetric and asymmetric stretching vibrations which are contributed to O-C=O and C–O. The weak band near 1623.24$cm^{-1}$is assigned to H–O–H bending vibrations mode [11].

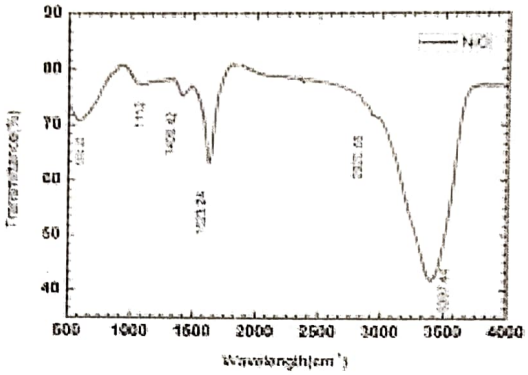

Figure-2. FTIR spectrum of NiO nanoparticles.

3.3. X-Ray diffraction (XRD) technique

The XRD pattern of prepared NiO nanoparticles depicted in **Figure-3**. The synthesized NiO nanoparticles exhibited crystalline structure with different diffraction angles. The diffraction peaks at 2θ= 31.42°, 36.51°, 52.59°associated with (111,200, 220) crystal planes. The peaks at (211,221) were occurred due to impurity or presence of plant extract components. The crystalline size of the NiO nanoparticles were determined by Debye Scherer's equation, i.e., $D=k \times \lambda/\beta \times \cos\theta$. The size of the nanoparticles calculated from Debye Scherer's equation was 3.37nm (33.7A°) with 2θ= 52.59° and the hkl plane was (220). The synthesized NiO nanoparticles were single crystalline having tetragonal cubic phase with a=b≠c. (JCPDS PDF No.: 73-1523) [12].

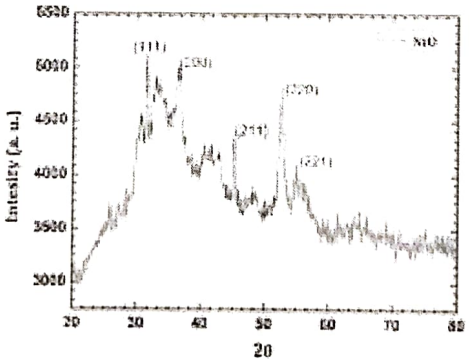

Figure-3. XRD pattern of NiO nanoparticles.

3.4. Antibacterial activity

The antibacterial activity of synthesized NiO nanoparticles was studied against microbial pathogens like Gram positive *Staphylococcus aureus* (*S. aureus*) and Gram negative *Escherichia coli* (*E.coli*). The simple agar well diffusion method was used

to study activity. The metal oxides like NiO enter into bacterial strain by diffusion mechanism and penetrate the cell wall of it. This penetration alters the structure of cellular membrane inner cellular components leads to cell death [13]. There are numerous mechanisms associated to antibacterial activity of NiO nanoparticles: - (i) generation of reactive oxygen species (ROS) which causes oxidative stress (ii) delocalization of membrane due to attachment of nanoparticles on bacterial cell membrane (iii) release of metal ions after binding to the bacterial membrane which was main cause of antibacterial activity [14]. Small particle size and minimum agglomeration effects on diffusion capacity and antibacterial efficiency of nanoparticles.The electrostatic interaction is present between negatively charged bacterial cell and positively charged nanoparticles.This interaction helps nanoparticles to enter into bacterial cell and may acts as bactericidal material.This entry of nanoparticle into cell generates reactive oxygen species leads to inhibition of bacterial growth. The increasing oxidative stress alters the structure of proteins, lipids, nucleic acids and stimulates the cell death [15].

In the current study, Gram positive *S. aureus* and Gram-negative *E. coli* were used to study antibacterial activity of NiO nanoparticles. After incubation of 24 hours the petri plates were observed for zone of inhibition. From the zone of inhibition, it was observed that NiO nanoparticles showed good antibacterial activity against Gram positive micro-organisms than Gram negative micro-organisms **Figure-4** and **Table-1** represented the information about antibacterial potential of NiO nanoparticles.

Table-1. Zone of inhibition by NiO nanoparticles.

Well no.	Sample	Bacterial strain	Zone of inhibition (mm)
1.	Control (50µl)	*S.aureus*	21±0.47
2.	NiO (50µl)		24±0.94
3.	NiO (100µl)		27±0.81
1.	Control (50µl)	*E.coli*	19±0.47
2.	NiO (50µl)		21±0.81
3.	NiO (100µl)		23±0.81

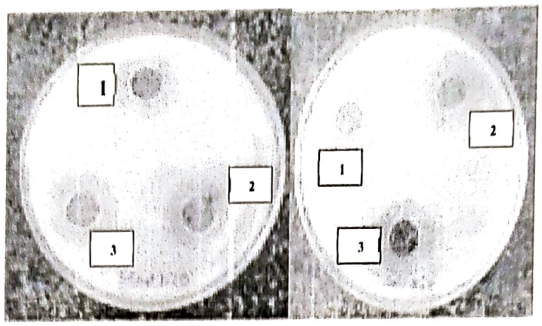

Figure-4. Zone of inhibition by NiO nanoparticles (a) *S.aureus*; (b)*E.coli.*

4. CONCLUSION

A novel green approach was carried out to synthesize NiO nanoparticles from tulsi leaves extract which has efficient reducing and capping properties. The structural properties were studied by using UV- visible spectroscopy, FTIR spectroscopy and X-ray diffraction technique. This work defines a specific procedure for the synthesis of NiO Nanoparticles based on cassava extracts. The antibacterial ability of NiO nanoparticles was studied against Gram positive and negative bacteria. It was found that, synthesized nanoparticles were potential antibacterial agent against Gram positive microbe than Gram negative. Thus, due to the eco-friendly and easy synthesis method, small size, single crystal nature and good antibacterial potential NiO nanoparticles can be used for various environmental and biomedical applications.

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