GREEN SYNTHESIS OF NICKLE OXIDE NANOPARTICLES FROM FICUS CARICA AND THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

Biomediated synthesis of nanoparticles has received much attention due to innovative and unique properties in various fields. In the present study, using *ficus carica extract*, nickel nanoparticles were synthesized. NiO-NPs were characterized using UV-visible spectroscopy, X-ray diffraction (XRD), energy dispersion X-ray (EDX) and scanning electron microscopy (SEM). X-ray diffraction confirmed the crystalline nature of the nanoparticles. SEM revealed the morphological features with a nanoparticle size of 20-25 nm. FTIR analysis confirmed nanoparticle capping by chemicals present in *ficus carica.* Later, EDX confirmed the elemental composition of the nanoparticles. The biosynthesized nanoparticles showed tremendous antibacterial activity against various bacterial strains. Overall, it was concluded that NPs played a vital role in various biological assays, but extensive in vivo and cytotoxic research is needed.

Keywords: *ficus carica,* NiO-NPs, biological applications. SEM, FTIR, XRD

INTRODUCTION

Nanotechnology represents the design, manufacture and application of materials at the atomic, molecular and macromolecular scale, to produce new nanometric materials (Hagens *et al.*, 2007). Nanotechnologies rely on a large scale taking into account the ability to bear blessings in regions as numerous as drug development, water purification, information and communication technologies and the production of more powerful and lighter materials (Benelmekki, 2015). Nanoparticles are generally defined as counting particles with at least one measurement that is much less than 100 nm. This definition puts them in a similar position in diverse area such as ultrafine debris (airborne particles) and places them as a subset of colloidal particles (Crane *et al.*, 2008). Un-designed nanoparticles clearly exist within the agglomerated level with different compositions, but the engineered nanoparticles only exist inside natural level (Buzea and Pacheco, 2017). Sources for securing common nanoparticles includes natural compounds (e.g. proteins, polysaccharides, viruses) in addition to inorganic compounds (e.g. iron oxy-hydroxides, aluminosilicates, metals) and are produced by weathering, volcanic eruptions, forest fires or microbial tactics (Lead and Wilkinson, 2006; Hough *et al.,* 2011). Different techniques for the synthesis of nanoparticles are mainly physical, chemical and biological synthesis and each of these techniques have their advantages and disadvantages in terms of throughput, scalability, particle length and distribution, shape uniformity and cost. Plant-mediated biosynthesis can be very easy, cheap and powerful manufacturing technique for nanoparticles. There is hindrance in maintaining a microbial culture due to infection while plant-mediated biosynthesis is an easy way and approach for large-scale nanoparticles fabrication without any infection (Kaur *et al.,* 2016).

Ficus carica, commonly called fig, is the deciduous plant belonging to the *Moraceae* family. It is native to South-west Asian countries and Mediterranean region (Lodhi *et al.*, 1969). The inside part of the fruit is a white inner ring containing a seminal mass bound with sweet gelatin. The seeds are numerous in variety and vary in size. The bark is smooth, silver-grey or gray (Badgujar *et al.,* 2014). Turkey Egypt, Morocco, Spain, Greece, California, Italy, Brazil and other places with often mild winters and hot, dry summers are the main manufacturers of figs [31]. Fruit can be consumed raw, dried, canned, or in other preserved forms (Neal, 1965). *F*. carica uses as traditional medicine for treatments of antispasmodic, cardiovascular, respiratory and metabolic disorders (Duke, 2002). The leaves, fruits and roots are used in indigenous medicinal system in different malfunctions gastrointestinal disturbances (cramps, indigestion, lack of food cravings and diarrhea), breathing (sore throat, cough and bronchial problems), and anti- inflammatory and cardiovascular problems (Burkill, 1935; Penelope, 1997). The figs were conventionally used for their healing benefits such as laxative, cardiovascular, respiratory and antispasmodic (Guarrera, 2005). *F. carica* fruit juice mixed with honey used for bleeding. In Indian medicines, the fruits used as a mild laxative, expectorant and diuretic (Solomon *et al.*, 2006). Due to high sugar content the fig as dried fruit is a good source of supplemented food for diabetic patients. It is commercialized in the market as sweet due to its high level of sugars (Veberic et al., 2008). Nickel oxide nanoparticles are very useful due to their non-complex nature of synthesis and important chemical characteristics (Zhang *et al.,* 2019; Alagiri *et al.,* 2012). NiO NPs can be synthesized using numerous tactics along with chemical bath deposition (Azaceta *et al.,* 2013), sol-gel way (Schoeberl *et al*., 2015), thermal evaporation (Ahmed *et al.,* 2014), pulsed laser deposition (Singh *et al.,* 2007), hydrothermal (Motahari*et al.*, 2015), inexperienced method (Ezhilarasi*et al.,* 2016) and spin coating (Xu *et al.,* 2005). Almost all of these approaches are complicated and have hazards including risk using natural solvents, expensive substance, unsafe

CV via the product, severe response limitation, longer time and so on (Raveendran *et al.*, 2003). NiO NPs have antibacterial, catalytic and biocidal properties as well as can be used in injury medicine, fuel sensors and solar cells (Ezhilarasi *et al.*, 2016). Currently NiONPs are manufactured using: exceptional physical and chemical procedures. That Law suitthey are not more practical luxury on a commercial scale, however Further goal some undesirable results on human existence and the environment and can result in cytotoxicity, carcinogenicity and genotoxicity, limiting its use for biomedical purposes(Anastas and Eghbali, 2010). So these problems must be resolved, and moves are required to augment an alternative solution for the production of NPs. that's why scientists evolved inexperienced chemical methods those are bigger durable, cleaner. currently, new trends was made in the synthesis of nanomaterials using characteristic biological active substances (microbes, algae, fungi, various)reduce and better plant life. This method is low-cost, simple, ecogreen, no risk, eliminate the need for high force, temperature and pressure and does not want to reduce, stabilize and limit overseas sellers(Mohamed *et al.*, 2019). inside of Gift studies, extracts from fresh leaves of *Ficus carica* were used to synthesize NiONPs.

Objectives: The objectives of the present study were the biosynthesis and characterization of NiONPs by Ficus Carica and to evaluate the antibacterial activity of synthesized NiONPs.

MATERIAL AND METHODS

Preparation of *ficus carica* (aqueous extract)

Ficus carica were collected in Charsadda, KP. The leaves were washed several times with tap water and then washed 3 times with double distilled water. The leaves were placed in ovens at 60ºC for 2 days. The leaves were cut using a grinder. 25 grams of *ficus carica powder* was dissolved in 250 ml of distilled water and kept in a water bath at a temperature of 60ºC for 2-3 hours. The extract was filtered with a fine large pore cloth and then filtered again through filter paper. After filtration it was stored for further experiments at 4 °C.

Preparation of the nickel oxide solution

To prepare the 0.1 M nickel oxide solution, 2 grams of nickel oxide was added to dissolve in 80 ml of distilled water and stirred for 30 minutes at 80 rpm in a magnetic stirrer. The prepared solution was covered with aluminum foil and stored at room temperature.

Synthesis of nickel nanoparticles

A 0.1 M aqueous solution was prepared of nickel oxide for nanoparticle synthesis. The *Ficus carica* extract was added to the aqueous solution of 0.1 M nickel oxide with vigorous stirring at 70°C for 2 hours on a hot plate until the color changed from dark orange to yellowish brown.

Characterization of nickel nanoparticles

UV Visible Spectroscopy

The main clue to the production of nickel nanoparticles using *ficus carica extract* was the color change from green to brown within 24 hours. Furthermore, characterization was carried out by UV-visible spectroscopy technique (UV-1650 PC Shimadzu). The reaction process was monitored between wavelengths from 200 to 800 nm by UV-visible spectra using nickel nitrate and *ficus carica* extract.

Fourier Transmission Infrared Spectroscopy (FTIR)

To remove unwanted impurities, centrifugation was taken place at 3000 rpm for 15 minutes of the suspension of nickel nanoparticles. The pellets were re-suspended in ethanol and centrifuged again at 10,000 rpm for 15 minutes for subsequent removal of impurities. The procedure was repeated twice. The final pellet obtained was washed with deionized water to obtain pure zinc nanoparticles. For further process the obtained sample was finally air dried at room temperature. The collected dust from niNPs was taken for FTIR, SEM and XRD analysis.

X-ray diffraction analysis

The crystalline structure of the nickel synthesized in green was confirmed by the XRD pattern. XRD data were recorded using PAN alytical X'pert X-ray diffractometer using cuk (wavelength = 1.54056).

Scanning electron microscopy (SEM)

Morphology and size were examined by SEM (JSEM-7600F, Japan) at a voltage of 20.0 kv. The synthesized niNPs were monitored morphologically using SEM (ZEISS-EVOMA 15, Japan). A small amount of nickel oxide nano particles were dropped on grid to get thin film of each synthesized sample.

Antibacterial Activity

media preparation

To prepare the medium, 8.4 g of nutrient agar was dissolved in 300 ml of distilled water and autoclaved at 121°C for 15 minutes. The medium was cooled to 40°C and then poured into autoclaved Petri dishes. The solidified media plates were then used for bacterial growth in a laminar flow hood.

Agar diffusion method for bacterial activity

NiO NPs synthesized and tested for antibacterial activity using *Ficus carica*, by the well diffusion method against *Escherichia coli*, *Citrobacter* , *Proteus vulgaris* and *B.subtilis.* Fresh twenty-four hour cultures of the bacterial pathogen were prepared on agar plates and standardized inoculum was used for the antibacterial assay. The NiO NPs solution was prepared at a concentration of 100 mg/ml. Four wells with a diameter of 8 nm were made in each plate and the synthesized NiO NP solution, in the concentration of 25 l, 50 l, 75 l and 100 l was loaded into each well. The zone of inhibition was measured after 24 hours incubation of plates at 37°C.

RESULTS

Ficus carica extract was used as a reducing agent for the preparation of NiO-NPs and featured the use of several advanced instruments. The prepared NiO NPs were analyzed by UV, XRD and SEM spectrophotometer. In addition, the prepared NiO-NPs were evaluated for various biological activities.

Analysis of NiO NPs by UV spectrophotometer

The synthesis of NiO Nps was initially confirmed by the color change to tan. The UV-Vis spectrophotometer also showed the absorbance at a wavelength of 301 nm, indicative of the formation of NiO nanoparticles (Fig. 1).

Analysis of Ni-NPs using FTIR

FT-IR characterization of NiONPs was performed to identify the molecules present in mushroom extracts, which are believed to be accountable for the decrease of Ni ions to nanoparticles and the capping reagent for the stability of the bioreduced nanometal. The FTIR measurement was performed using the obtained spectra in the range of 500 to 4500 cm^{-1} overf. Ni nanoparticles showing the absorption band of biosynthesized NiO nanoparticles were observed at 1416, 1135,877 and 629 nm in the FTIR spectrum. The absorption band corresponding to 1416 cm -1 was due to OH, carboxylic acid stretching vibrations and hydroxyl stretching vibrations. Furthermore, 1135 cm-1 representing CO, plus 877 cm-1 CH and 629 C-Br which were sharper and wider for NiO nanoparticles, participate in the reaction (Fig. 2).

NiO nanoparticle analysis with SEM

SEM analysis was performed to visualize the shape and size of the nanoparticles. SM6510LV A scanning electron microscope was used to determine the shape of NiO NPs from *Ficus carica* (Fig. 3). SEM images were viewed at different magnification ranges, such as 10 m-200 nm and 20 m, which clearly demonstrated the presence of spherical nanoparticles with a mean mean diameter of 20-25 nm **.**

Energy Dispersive X-rays (EDX)

The analysis element composition of NiO NPs synthesized in aqueous medium was examined by energy-dispersive X-ray spectroscopy, as shown in Fig. 4. The EDX composition showed a strong peak at 3 keV, confirming the success in the synthesis and purity of nanocrystalline ZnO NPs. The presence of oxygen clearly confirmed the presence of metal oxide nanoparticles.

X-ray diffraction analysis

XRD Spectra provides insight into the crystallinity of the nanoparticle representing XRD Spectra of NiO NPs synthesized using fresh Carissa ficus leaf extract (Fig. 5). The mean particle size was calculated by Scherrer's formula = 0.9xλ/d CosΘ, where λ was the wavelength, X-rays, β was the full width at half maximum (FWHM) of the peaks at the diffraction angle. Di X-ray response peaks at 2°o 42.52, 62.14, 74.5 were identified as NiO cubic phase crystallite peaks with different diffraction planes [200], [220]. The other peaks found at an angle (2θ) of 30.92, 65.4 were identified as Ni 2 O 3 corresponding to [002]. A peak observed at (2θ) < 44.66 was identified for Ni [111]. The about 25nm size was found to be as an average. Therefore, this result confirmed the formation of nickel oxide nanoparticles and some additional peaks showed the impurity.

Antibacterial activity

The antibacterial effect of NiO-NPs against bacterial strains *Citrobacter* , *Proteus vulgaris*, *Escherichia coli* and *B. subtilis*, using the well diffusion method, performed at different concentrations, namely 25, 50, 75 and 100 mg/ml. NiO-NPs were found to be more effective against selected bacterial strains. The antibacterial activity of NiO-NPs synthesized at different concentrations is shown in Figure 6 and Figure 7. NiO Nps showed greater activity against *Citrobacter* , *E.Coli* , *B. subtilis* and *Proteus vulgaris* , shown in Figure 7. Maximum zone of inhibition (18.5 mm) was observed against *Citrobacter* followed by *E.Coli* (15.5 mm), while against *B.subtilis* and *p.uvlgairs* the activity was (16 mm) and (14.5 mm) respectively in 100 L ZnO Nps solution. the added 75 µl Nps solution shows the zone of inhibition of 15.5 mm against *Citrobacter* , 15 mm against *E.coli* , 13 mm against *B.subtilis* and 13.5 mm against *p.uvlgaris.* in the 50 l respectively (13 mm, 11 mm, 13 mm and 12 mm) in the 25 l (10.5 mm, 8.5 mm, 9.5 mm and 11 mm).

DISCUSSION

Nanotechnology is applicable in different fields of technological knowledge and era because the many blessings over non- organic products systems, various companies use biological systems for the production of nanoparticles. most extraordinary microbes used for nanoparticle synthesis, fungi are effective candidates for the fabrication of intra- and extracellular metal nanoparticles. In the present study, NiO nanoparticles were synthesized from Ficus carica extract. specific bacterial properties used to be explained by means of different comparable studies such as Penicillium sp (Hemath *et al.*, 2010), Amylomyces roux pressure KSU-09 (Musarrat *et al.*, 2010), Penicillium brevicompactum WA 2315 (Shaligram *et al.*, 2009) Aspergillus fumigatus (Bhainsa *et* *al*., 2006). The main finding of this study is characterization of NiO nanoparticles with help of specifically strategies, that is UV (UV Vision Spectroscopy), Fourier Rework Infrared Spectroscopy (ft -IR), and Scanning Electron Microscope (SEM). The formation of NiO NPs seen by UV spectroscopy through color conversion from dark orange to tan. UV- visible spectroscopy has been widely used to locate the presence of nickel nano debris for the duration of green synthesis and height of NiO NPs synthesized in aqueous extract medium at 301 wavelength (nm).

FTIR spectra of biosynthesized nickel nanoparticles were recorded to pick out the capping and green stabilization of the metal nanoparticles by using biomolecules found in coriander leaf extract. The feet-IR characterization of NiONPs turned into carried out. It becomes completed to identify the molecules found in plant extracts thought to be responsible for the discount of Ni ions to nanoparticles and the capping reagent for the stability of this bio reduced Nano metallic. FTIR dimension was accomplished the spectra received turned into between 500 to 4500 cm $^{-1}$ oVERf Ni nanoparticles which confirmed the absorption band of biosynthesized NiO nanoparticles were observed at 1398, 887,635 and629 inside the FTIR spectrum. The absorption band similar to 1398 cm-1 was because of C-H, stretching vibrations of carboxylic acid and hydroxyl stretch vibrations. in addition, 887 cm-1 representing to C -CH2, similarly 635 cm-1 CH=CH and 629 zero-H which are sharper and broader for NiO nanoparticles participates inside the response. therefore, the synthesized nickel nanoparticles had been surrounded via proteins and metabolites consisting of phenolic acid, carboxylic acid, flavonoids. This indicates that the organic molecules could probably carry out dual functions of formation and stabilization of Ni nanoparticles in aqueous medium (Vasudeo *et al* 2016). within the cutting-edge study the synthesized zinc nanoparticles showed high-quality antibacterial pastime. The antibacterial impact of NiO-NPs towards bacterial strains Citrobacter, Escherichia coli, Proteus vulgaris and B. subtilis the usage of well diffusion method done at exceptional concentrations i.e. 25, 50, 75 and 100 mg/ ml. It became located that NiO-NPs were best against decided on bacterial strains. NiO Nps confirmed highest hobby towards Citrobacter, E.Coli, B. subtilis and proteus vulgaris proven in desk 1.maximum quarter of inhibition (18.5mm) become found towards Citrobacter followed via E.Coli (15.5mm) even as towards B.subtilis and p.uvlgairs the hobby turned into (16mm) and (14.5mm) respectively turned into seen in 100 µl of ZnO Nps answer. the 75 µl delivered Nps answer shows the region of inhibition 15.5mm against Citrobacter ,15mm against E.coli,13mm towards B.subtilis and thirteen.5mm in opposition to p.uvlgaris.in the 50µl respectively confirmed (13mm,11mm,13mm and 12mm) in 25 µl (10.5mm,eight.5mm,9.5mm and 11mm). The presences of NiO NPs were showed from the EDX of the nanoparticles. The EDX composition confirmed a robust peak at 3 keV affirmed the a hit synthesis and purity of nanocrystalline NiO NPs. The presence of oxygen actually confirmed the presence of metallic Oxide nanoparticles. within the present observe the SEM snap shots display that zinc nanoparticles synthesized in are abnormal uniform and homogeneous in morphology and the particles are non-agglomerated and properly dispersed. the scale of the debris was additionally calculated by using the use of photograph j software program which become 72nm. The presences of NiO NPs had been showed structure and particle length of Nickel Oxide nanoparticles were acquired by way of X-ray diffraction (Rigaku Miniflex) using Cu-kα radiations (λ= 1.5406 Ǻ) in 2θ range 20 to eighty. The average size of the debris changed into calculated by the Scherrer's formula: where λ is the wavelength of X-ray radiation, β is the overall width at 1/2 most (FWHM) of the peaks on the diffracting angle θ. The X-Ray diffraction peaks at 2θ o forty two, fifty two, sixty two.14, 74. five have been identified as peaks of NiO cubic phase crystallites with various diffraction planes [200], [220], [311]. The alternative peaks observed at an attitude (2θ) o 30.92, 65.four were diagnosed as Ni2O3which corresponds to [002], [004]. A peak located at (2θ) o 44. sixty-six turned into diagnosed for Ni [111] [10]. The common length was located to be round 25 nanometer (nm). therefore, this result showed the formation of Nickel Oxide nanoparticles and some extra peaks suggests there's some impurity.

CONCLUSION

It was concluded from the present study that the bio-assisted method of nanoparticles is a good alternative compared to the dangerous physico-chemical method. XRD confirms that a nanoparticle is crystalline. The FTIR study confirmed the change of metal ions in nanoparticles. SEM and EDX revealed the morphological properties and elemental composition. The results indicated the effectiveness of the nanoparticles against various bacterial and fungal strains. The small size of NPs plays an important role in biological assays and it has been detected that smaller nano particles have more effective activities. Current findings show that NPs can be used for numerous biomedical applications and cytotoxic research, but more research is needed to fully explore the innovative and remarkable ability of NPs in nanomedicine.

CONFLICT OF INTEREST

The authors have no conflict of interest.

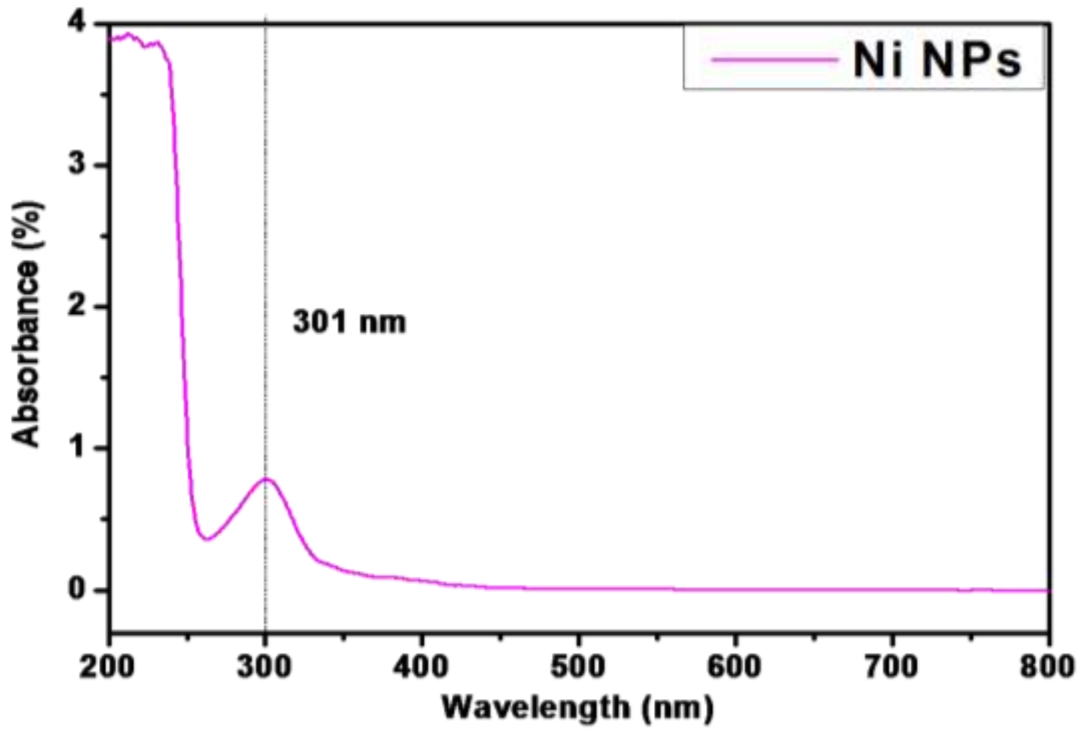

Figure 1: UV Visible Spectrum of NiO NPs

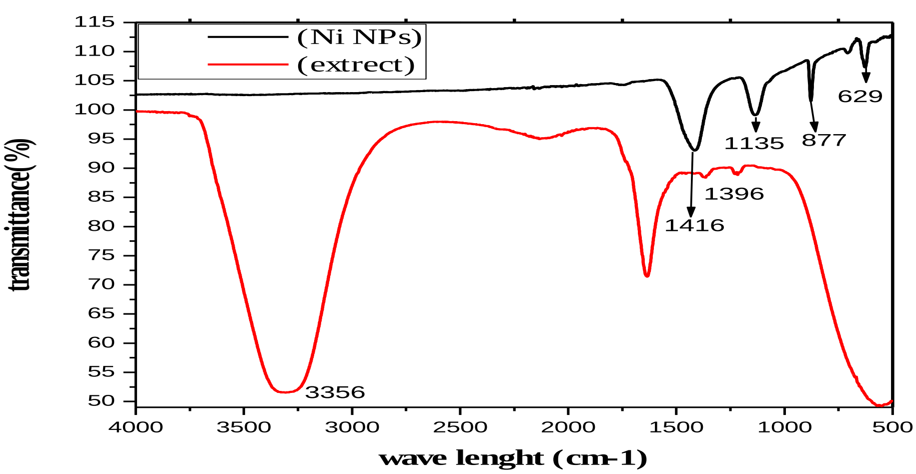

Figure 2: FT-IR spectrum

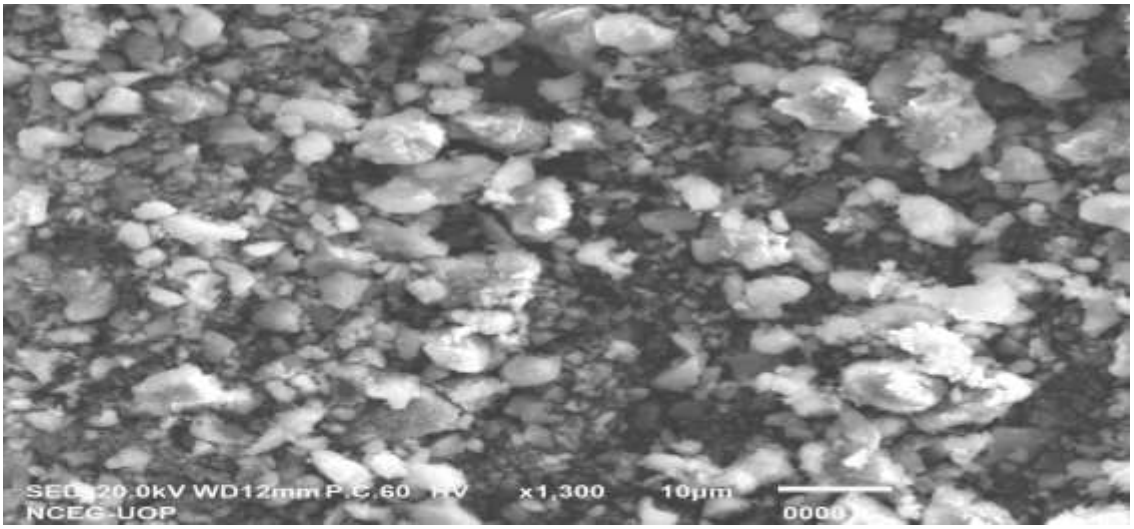

Figure 3: SEM images of prepared NiO nanoparticles.

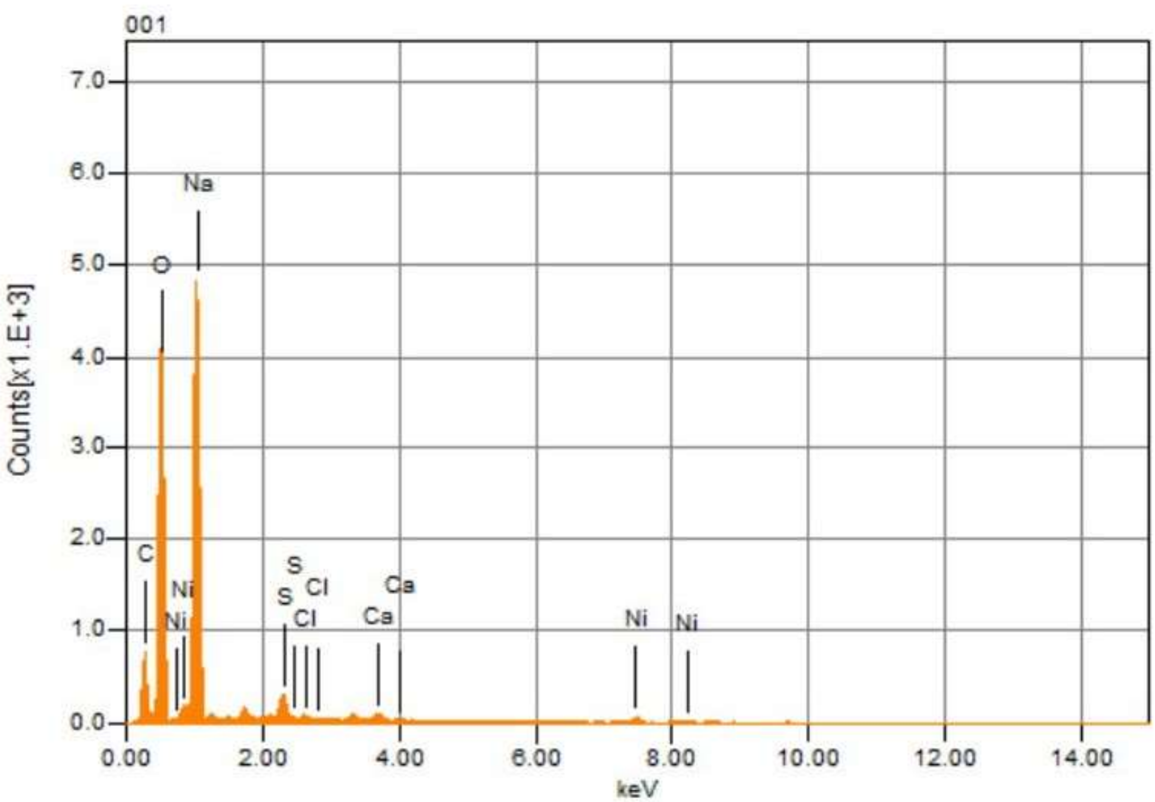

Figure 4: EDX figure of prepared NiO nanoparticles.

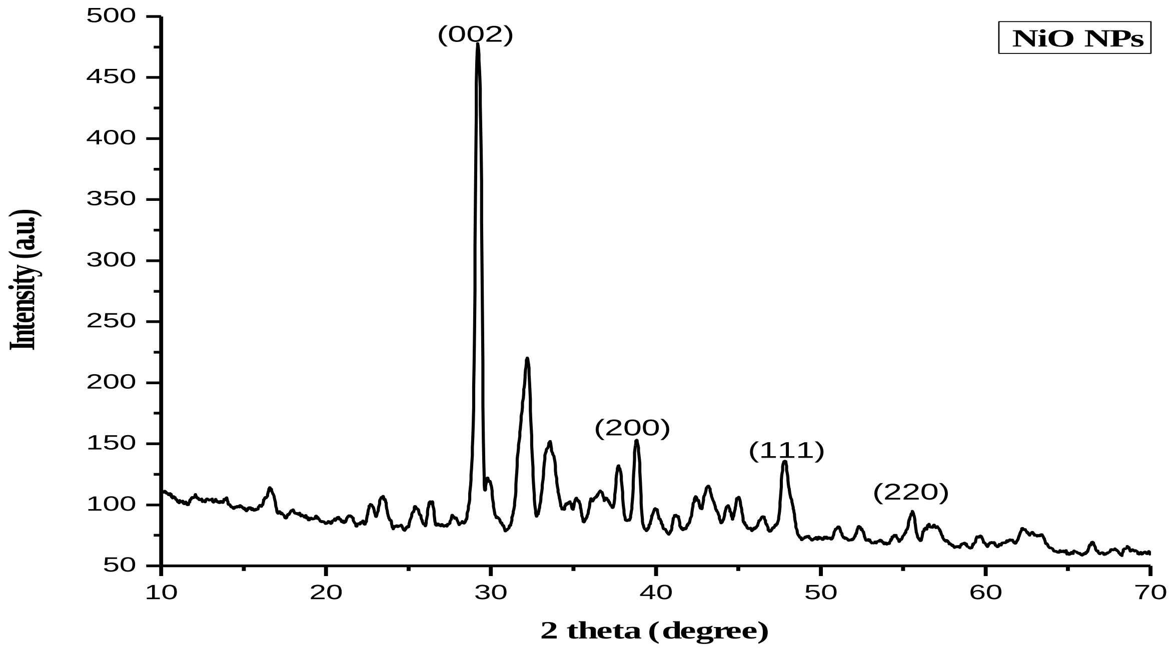

Figure 5: XRD Chart

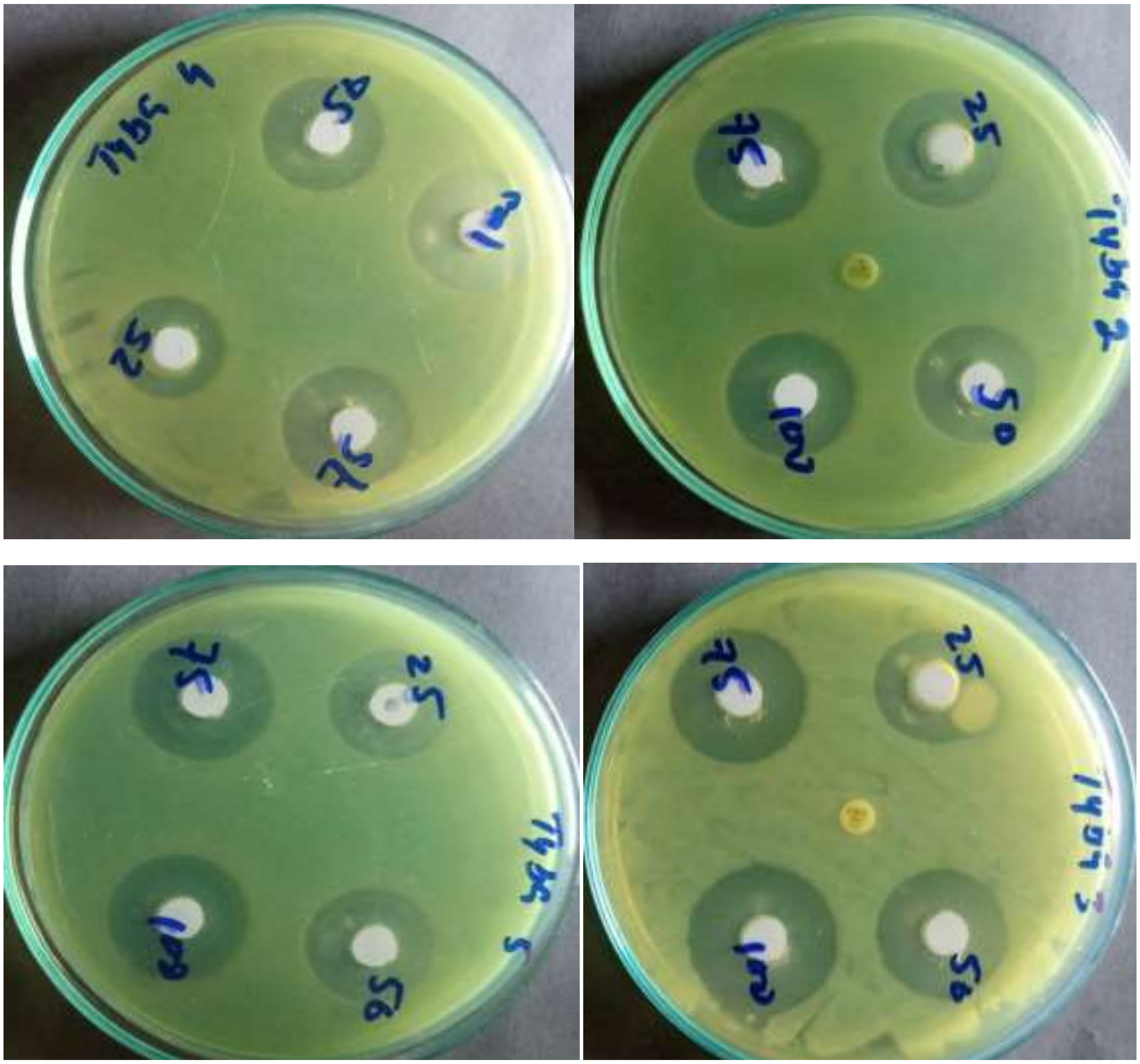

Figure 6: Antibacterial Activities of NiO Nps against Selected Bacterial Strains

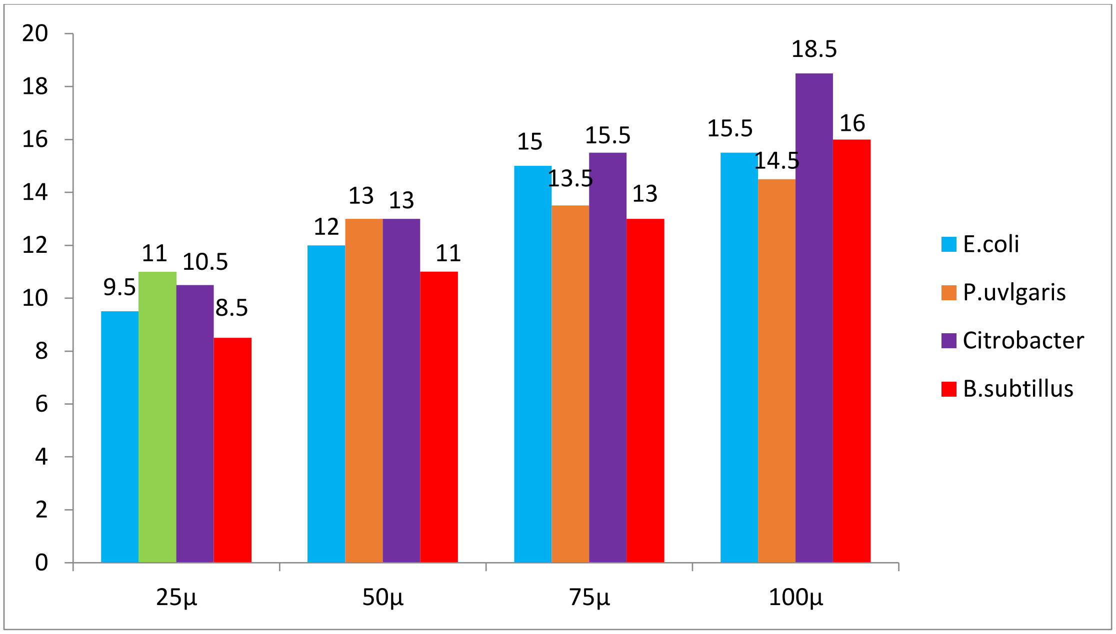

Figure 7: Antibacterial activity of NiO NPS against selected bacterial strains.

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