GREEN SYNTHESIS, CHARACTERIZATION AND APPLICATIONS OF NICKEL OXIDE NANOPARTICLES USING FRUIT EXTRACT OF TRIBULUS TERRESTIS

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ABSTRACT

Nickel oxide nanoparticles were successfully synthesized by using a simple and green synthetic route by using metal salt and fruit extract of Tribulus terrestris which act as reducing and stabilizing agent .The synthesized nickel oxide nanoparticles were characterized by using various analytical techniques such as UV-VISIBLE DRS, FT-IR, XRD, EDAX, FESEM, TEM. Biological test results exposed that synthesized nickel oxide nanoparticles have broad spectrum antimicrobial activities against Escherichia coli, Bacillus subtilis, Aspergillus niger and cytotoxic activity on MCF-7 cell line.

***Keywords* :** Nickel oxide nanoparticles, Tribulus terrestris, green synthesis, antimicrobial activity.

INTRODUCTION

Nickel oxide nanoparticles with a uniform size and well dispersion are desirable for many applications in designing ceramic, magnetic, electro chronic and heterogeneous catalytic materials [1]. Nickel oxide became one of the most important transition metal oxide [2-4]. Most of these applications require particles with a small size and a narrow size distribution [5]. With the volume effect, the quantum size effect and the surface effect, nickel oxide nanoparticles are expected to possess many improved properties and even more attractive applications than those of bulk sized nickel oxide particles [6-8]. Nickel oxide nanoparticles is a p-type semi conductor transition metal oxide with cubic lattice structure. These nanoparticles are studied intensively and attracted increasing attention due to their electron transfer capability, electro catalysis and super capacitance properties.[9-10].

Several techniques have been developed for the preparation of nickel oxide nanoparticles, such as sol-gel [11], surfactant-mediated synthesis [12], thermal decomposition [13], polymer-matrix assisted synthesis [14] , spray-pyrolysis[15], ultrasonic radiation, pyrolysis by microwave, hydrothermal synthesis, precipitation calcination method, carbonyl method, laser chemical method, micro emulsion method and combustion[16-21].

These methods used for synthesis of nickel oxide nanoparticles are expensive and harmful to environment due to involvement of various hazardous chemicals responsible for many health problems.

In recent, green synthesis of nanoparticles was achieved by using plant extract due to its low cost, easily available, non toxic, biodegradable and environment friendly characteristics.

In this work, green synthesis of nickel oxide nanoparticles using fruit extract of Tribulus terrestris as reducing and capping agent. Tribulus terrestris is found to be growing in subtropical areas around the world commonly known as Gokhru belonging to the family Zygophyllaceae widely distributed throughout India. Phytochemical studies have shown that this plant contains biologically rich compounds such as steroids, saponins, flavoniods, alkaloids and unsaturated acids which were involved in promoting numerous physiological responses [22]. Tribulus terrestris has been shown to exhibit diuretic [23] , anti-urolithiatic [24] , central nervous system stimulant [25] , antimicrobial [26] , antifungal activities [27] , antioxidant and antihypertensive activity in rat heart [28-29].

Fruits of T. terrestris.

Leaves and flowers of T. terrestris

The use of non toxic materials like plant extract for the synthesis of nanoparticles offers numerous benefits of pharmaceutical applications..The effect of metal oxide nanoparticles on antibiotics has been studied preservation in mind the fact that nickel oxide nanoparticles have an intrinsic bactericidal effect of their specis. The synthesized nickel oxide nanoparticles are characterized by UV-VISIBLE, X-RD, FT-IR, FESEM, EDAX,TEM analysis. The antimicrobial activity of metal oxide nanoparticles is well known. Hence we create use of this property to inhibit the growth of Bacillus subtilis, Escherichia coli and Aspergillus niger using disc diffusion method. Synthesized nanoparticles are targeted as drug delivery, anticancer agents and antibacterial activity . Bacillus subtilis, Escherichia coli and Aspergillus niger strains are selected as they are highly contagious, then we can assess the potential antimicrobial activity of nickel oxide nanoparticles.

EXPERIMENTAL METHOD

Reagents

Nickel chloride hexahydrate, Sodium hydroxide (All the chemicals used in this were AR grade purchased from Sigma Aldrich, India) , double distilled water.

Preparation of Tribulus terrestris Fruit Extract

The fruits of Tribulus terretris were collected from Andhra university,Visakhapatnam. The fruits were rinsed with water several times followed by double distilled water to remove the dust particles and then the fruits were dried under direct sun light for two weeks to completely remove the moisture.

The dried fruits were pulverized well with mortar and pestle to make the powder. 10 gr of fruit powder was mixed in 100 ml of double distilled water and the mixture was heated at 80^0 c for 30 min.Then the boiled extract was cooled at room temperature and filtered using whatman no.1 filter paper and the filtrate was stored at 4^0 c for further usage.

Green Synthesis of Nickel oxide Nanoparticles from the Fruit Extract of Tribulus terrestris

10 ml of Tribulus terrestris fruit extract is added to a 90 m1 of 1mmol nickel chloride solution and then boiled at $80\text{-}100^0$ c for 45 min by using a stirrer-heater. Followed by addition of 10ml of 1M NaOH drop wise to maintain alkaline pH (11). Then the mixture was boiled until it converted to a green colour precipitate. This precipitate is then taken out and washed repeatedly for three times with double distilled water followed by ethanol to remove impurities. The precipitate is then taken out in to a clean ceramic crucible and calcinated in an air heated furnace at 300^0 c for 3 hours. A green coloured powder was obtained and this powder was collected carefully and then grind with mortar and pestle for uniformities of the powder.

CHARACTERIZATION

The synthesized nickel oxide nanoparticles were characterized by using a UV-VISIBLE DRS in the wavelength range between 200-800 nm. The FT-IR absorption spectra was recorded on a Perkin-Elmer

GX FTIR system used to obtain16 cm^{-1} resolution spectra in the range 400 to 4000 cm^{-1} (absorbance mode).

The crystalline structure of the nickel oxide nanoparticles were measured by using a Bruker D8 Advance X-ray diffractometerwith CuKα radiation of wavelength λ = 1.54056 Å. The X-ray diffraction (XRD) measurements were carried out in the locked coupled mode in the 2θ range of 20 to 80.

The surface morphology and composition of nickel oxide nanoparticles were investigated by FESEM (Field Emission Scanning Electron Microscopy) developed by Carl Zeiss. An accelerating voltage of 15 to 19 keV and probe current of ~800 pA. The shape and size of the nanoparticles were obtained by using transmission electron microscopy(TEM).

RESULTS AND DISCUSSIONS

UV-Visible Diffuse Reflectance Spectrum (UV-DRS)

UV-Visible Spectroscopy absorption peak means the electrons are absorbing the energy at 200-800 nm wavelength. Electrons are absorbing energy means the electrons are going to excited state from its ground state. Electrons are going to excited state from its ground state means the material is having band gap, thus which can be determine by absorption wavelength. The electronic absorption spectra of metal oxides were recorded in DMF in the range 200–800 nm. Diffuse reflectance spectral studies in the UV-Visible-NIR region were carried out to estimate the optical band gap of the synthesized nanoparticles

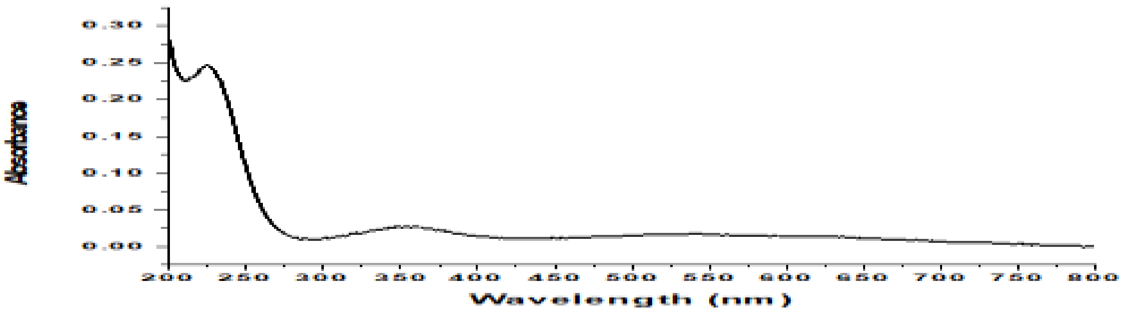


Fig.1. UV-Visible diffuse reflectance spectrum of nickel oxide NPs

Fig.1. Shows the absorption spectrum of the nickel oxide nanoparticles excited at 360 nm which corresponds to the nickel oxide nanoparticles. The purity of the nickel oxide nanoparticles confirmed by the non existence of the other absorption peaks in the spectrum. The excitation peak corresponds to the band to band transition which also confirms the blue shift in the band gap of nickel oxide nanoparticles.

The band gap estimated for this sample 3.44eV that is slightly higher than that of bulk nickel oxide. This blue shift may be attributed to quantum confinement effects.

Fourier-Transform Infrared spectrum (FT-IR)

Fig.2. Shows the FT-IR spectrum of the nickel oxide nanoparticles synthesized by green method, which is acquired in the range of 250-4000 cm^{-1}.

The band between the 450-600$cm^{-1}$is correlated to metal oxide bond (NiO). The peaks in the range of 470 cm^{-1} indicates the presence of NiO (Ni-O bond). The peaks located at 1031cm^{-1} represents C-N stretching vibrations of amines. The peaks located at 1581 cm^{-1} represents the aromatic ring (c=c) stretching..The absorption peak at 2995 cm^{-1} is due to C-H stretching of aliphatic groups. The broad and intense peak at 3402cm^{-1} is due to O-H Stretching.

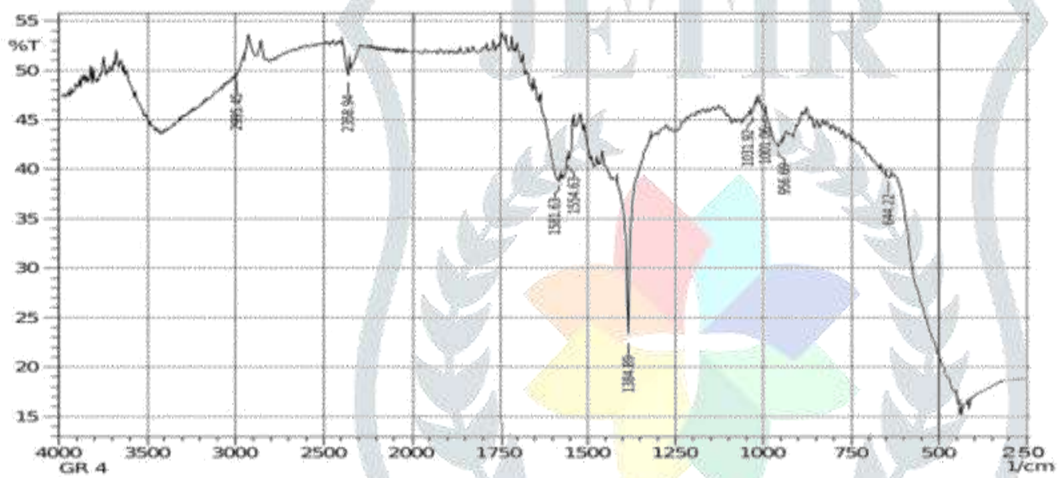


Fig.2. FT-IR Spectrum of nickel oxide nanoparticles

X-Ray Diffraction (XRD)

The XRD results are shown in fig.3.The XRD peaks match with that reported for nickel oxide. The peaks are indexed as (100), (002), (101), (110), (200), (112),and (004) and when 2θ is varied from 10° to 80°(31.48º(540),37.1º(680), 45.08º(420), 59.44º(580),65.51º(725) and 77.51º(215) respectively.All the peaks in the figure match well with the standard JCPDS pattern for NiO (JCPDS card 47-1049).

The peaks are sharp indicating good nanoparticles of the product. The broadness at the bottom of the peaks shows that the product is of small size. No other peak other than nickel oxide is observed indicating that the product is pure nickel oxide without any impurities or unreacted precursor. Average particle size of nickel oxide nanoparticles is found to be 85 nm using the Debye–Scherrer's formula.

Diffraction pattern corresponding to impurities are found to be absent. This proves that pure nickel oxide nanoparticles are amorphous nature.

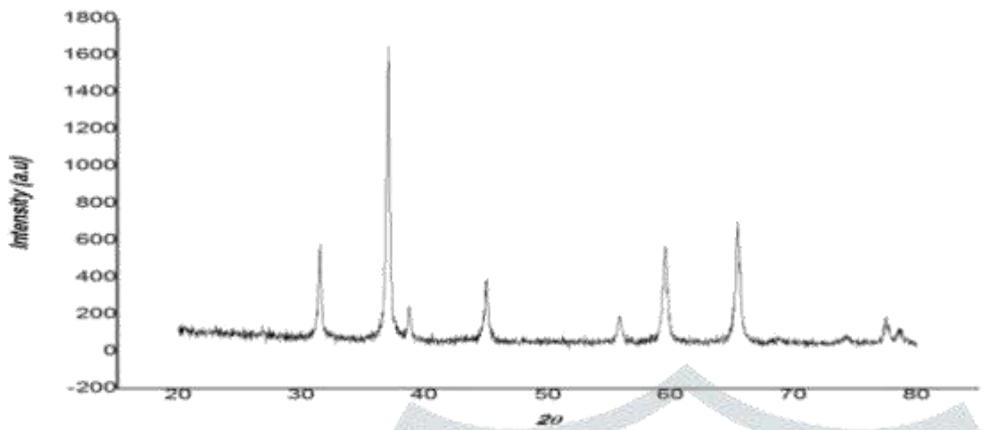


Fig.3. XRD pattern of nickel oxide nanoparticles

Field Emission Scanning Electron Microscope (FESEM) Analysis

FESEM analysis was used for the morphological study of nickel oxide nanoparticles calcinated at 300°c is shown in Fig.4. It can be seen that the particles adopt irregular morphology with different sized particle. In addition, nickel oxide nanoparticles show spherical rod shape with smooth surface. It clearly indicates the fine rod like particles adsorbed on the surface due to the agglomerates. Nanoparticles size is in the range of 20-50 nm in diameter. The FESEM image shows nickel oxide nanoparticles are sherical rod shape with smooth surface and the size of the particles around 100 nm

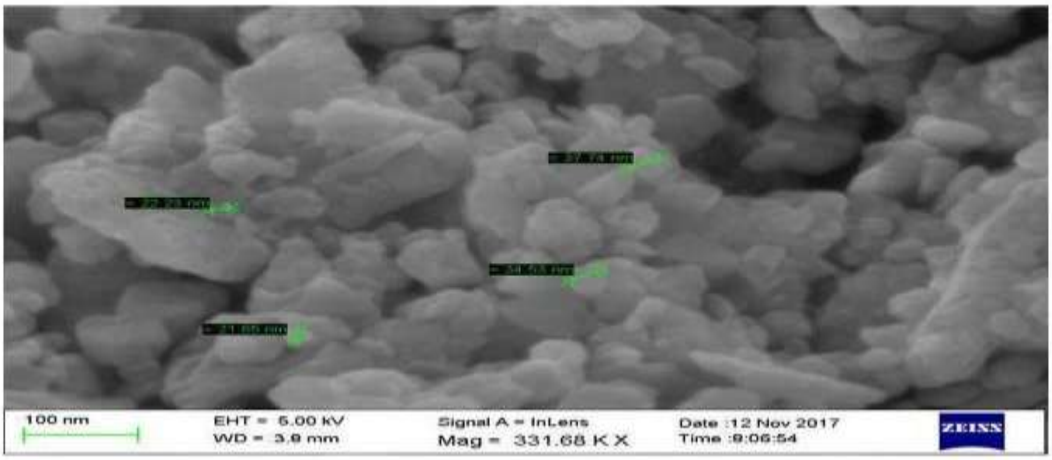
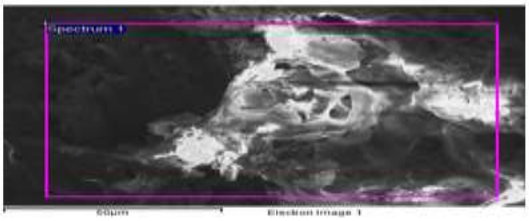


Fig.4. FESEM image of nickel oxide nanopartricles

EDAX Analysis

Element	Weight%	Atomic%
O K	52.66	80.90
Ni K	47.34	19.09
Totals	100.00	



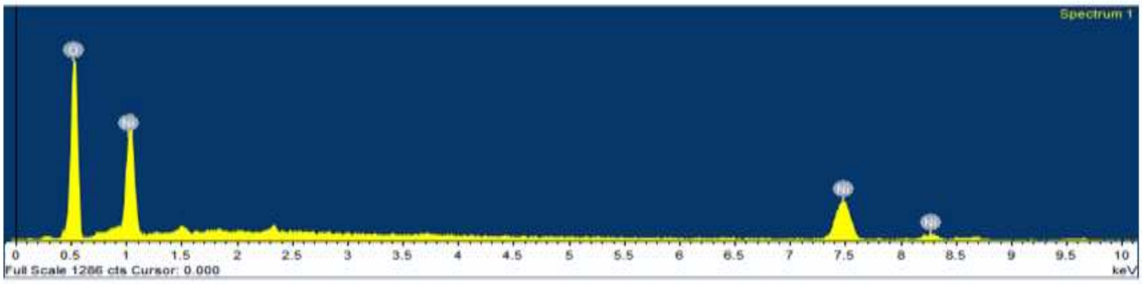


Fig.5. EDAX image of nickel oxide nanoparticles

The quantitative compositional analysis of nickel oxide nanoparticles synthesized from Tribulus terrestris fruit extract is carried out using Energy Dispersive X-ray (EDAX) Spectroscopy measurement. The EDAX analysis also confirms the presence of Nickel oxide nanoparticles (Fig.5). From EDAX Spectrum the composition of elements such as a nickel (47.34%) and oxygen(52.66%) are identified.

Transmission Electron microscope (TEM) Analysis

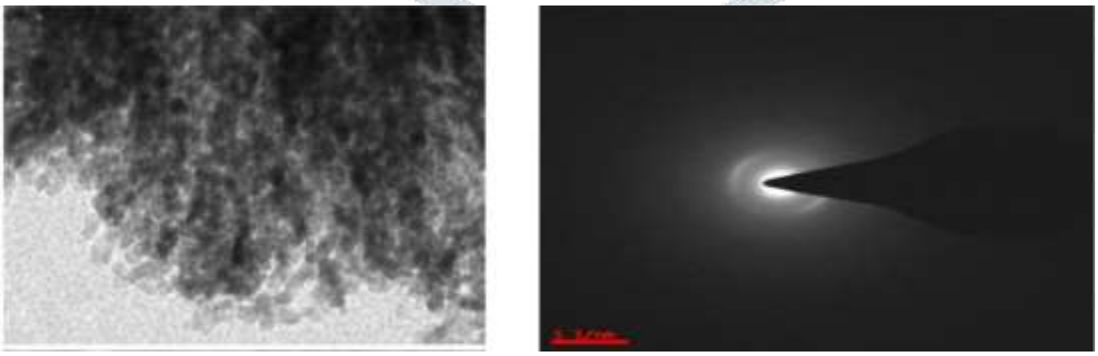


Fig..6. TEM image of nickel oxide nanoparticles

Fig..6. Shows the TEM image of nickel oxide nanoparticles synthesized from Tribulus terrestris fruit extract. TEM analysis is carried out to confirm the actual size of the particles, TEM images gives nanoparticles nature, morphology and orientation of the nickel oxide nanoparticles and illustrated in fig. The size of the particle is around 50 -100 nm.

Antimicrobial Screening of nickel oxide nanoparticles

The nickel oxide nanoparticles is screened in vitro for antibacterial activity against Escherichia coli, Bacillus subtilis and antifungal activity against Aspergillus niger by Agar-well disc diffusion method. The antibacterial and antifungal activities of nickel oxide nanoparticles are listed in table.1.

Fig.7. Inhibition zones for nickel oxide nanoparticles against B.subtilis, E.coli

Fig.8. Inhibition zones for nickel oxide nanoparticles against A.niger

Table.1. Antimicrobial activities of nickel oxide nanoparticles

Bacteria	**Inhibition zone (mm)**
E.coli	18
B.subtilis	16
Fungi	**Inhibition zone (mm)**
A.Niger	6

The nickel oxide nanoparticles showed good antibacterial activity against E.coli, B.subtilis and Antifungal activity against A.niger.

Cytotoxic studies of Nickel oxide nanoparticles

The synthesized nanoparticles is screened for their cytotoxicity (MCF-7, cell lines). From the data, it is observed that the nanoparticles displayed their cytotoxic activities as IC_{50} (μg/mL) against breast cancer MCF-7 cell line.

Table.2 Dose response of nickel oxide nanoparticles on MCF-7 cell line

Conc (µg/ml)	% cell survival	% cell inhibition
0.1	90.3	9.7
1	94.50	5.50
10	63.47	36.53
100	10.58	89.42

The above table details the high potent nature of nickel oxide nanoparticles. The cell survival is decreasing and percentage of cell inhibition is increasing with the increasing concentration of the nickel oxide nanoparticles. The IC_{50} value for nickel oxide nanoparticles nanoparticles is about 31..95µg/mL.

IC50	31.95µg/mL

Fig.9. Effect of nickel oxide nanoparticles on MCF7 cell viability for 24hrincubation time

CONCLUSIONS

Synthesis of nickel oxide nanoparticles is carried out using fruit extract of Tribulus terrstris. Very versatile, nontoxic and eco-friendly approach for the synthesis nickel oxide nanoparticles is presented in this chapter. Nickel oxide nanoparticles have been synthesized successfully by using a green method. The nanoparticles are characterized for structure and morphology using UV-VISIBLE, FT-IR, XRD, SEM, EDAX, TEM.

UV-Visible Diffuse Reflectance Spectrophotometer gives absorption maximum at 360 nm and having band gap 3.44 eV for nickel oxide nanoparticles. FT-IR Spectra gives the peak in the range of 470 cm^{-1} indicates the presence of NiO (Ni-O bond). FESEM shows nickel oxide nanoparticles size is below 100 nm. EDAX analysis of the samples showing Ni and O present in the samples . XRD pattern shows the phase nature and crystalline size. Which is done by particle size is calculated by using by Debye-Scherrer's formula. TEM image exhibits as synthesized nickel oxide nanoparticles prepared by green method with an average diameter of 50-100 nm.

Nickel oxide nanoparticles are screened in vitro for antibacterial and antifungal activity by disc diffusion method. The bacterial organisms used in this study are Escherichia coli, Bacillus subtilis and fungal organism is Aspergillus niger. The observed inhibition zones for nickel oxide nanoparticles are in the range of 18 mm for E.Coli, 16mm for Bacillus Subtilus and 6 mm for A.Niger. The screened data in these reports are in good agreement with the previous data and the inhibition zone images are pictorially recorded. The cytotoxic activities of nickel oxide nanoparticles screened by MTT assay. We have screened for one type of cancer cell line, viz., MCF-7 (breast cancer), nickel oxide obtained IC_{50} values in the range of 25- 35μg/ml for MCF-7 cell line most of these nanoparticles are in cytotoxic activity. In some cases, the IC_{50} values are less than the first metal based drug cisplatin.

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