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Green synthesis and characterisation of NAT loaded zinc oxide nanoparticles

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ABSTRACT

The present study states a green approach for the synthesis of zinc oxide nanoparticles employing methanolic leaves extract of *Nyctanthes arbor-tristis*. Leaves extract was used as the biological reduction agent for synthesizing zinc oxide nanoparticles from zinc acetate dihydrate. Metal oxides such as ZnO have received increasing attention in recent years because of their stability under harsh processing conditions, and also because they are generally regarded as safe materials for human beings and animals. Use of plant and plant materials for the synthesis of Zinc nanoparticles is relatively new and exciting research field. Various plants were used for the synthesis of nanoparticles using green synthesis method. Green method of synthesis of nanoparticles is easy, efficient, and eco-friendly in comparison to chemical-mediated or microbe-mediated synthesis. Since, green synthesis is the best option for the synthesis of nanoparticles. The prepared nanoparticles of Zinc oxide were characterized by using XRD, FTIR, UV-VIS Spectroscopy, TEM and SEM. The objective of this review was to report on the synthesis of Zinc oxide nanoparticles by using different plant extracts and their significance in different fields.

INTRODUCTION

Nyctanthes, also known as Harsingar, is an important member of Ayurveda, the traditional Indian medicine science. It is blessed with a diverse spectrum of medicinal properties, such as anti-helminthic, antimicrobial, antiviral, antileishmania, anti-allergic, anti-diabetic and anti-cancerous. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic11. Nanoparticles hold extraordinary and attractive properties due to their small sizes, large surface area, free hanging bonds and superior reactivity. Nowadays, nanotechnology has a vast range of application in diagnosis, drug delivery, food industry, paints, electronics, sports, environmental cleanup, cosmetics, and sunscreens. Green synthesis approaches of herbal extracts are gaining interest towards treatment of various diseases. Recently, plants and their extracts based nanoparticles synthesis were considered to be the best techniques

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because of easy availability, mass production and eco-friendly process. Zinc (Zn) an essential micronutrient that exhibits antioxidant properties and protects cardiac cells against different oxidative stressors13. According to some previous studies, Zinc oxide nanoparticles (ZnO NPs) are found to be non-toxic, biosafe, biocompatible making them an ideal candidate for biological applications. The plant *Nyctanthes* extracts have been reported to yield gold, silver and titanium dioxide Nanoparticles. Two majorly researched substrates for biosynthesis of ZnONPs are zinc acetate and zinc nitrate. This is, to the best of our knowledge, the first study reporting synthesis of zinc oxide Nanoparticles using leaf extract of *Nyctanthes arbor-tristis* and zinc acetate [1].

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METHODS

Collection and authentication of plant material

Leaves of *Nyctanthes arbor-tristis* were collected from in and around the region of Namakkal, Tamilnadu, in the month of November. The plant was authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen is preserved in our laboratory for future reference [2, 3].

Preparation of plant extract

The plants material was shade dried at room temperature. The dried plant materials were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve no. 40 was used for extraction. Powdered plant material (500 gm) was extracted with 80% methanol at room temperature for 72 hrs. The extract was filtered and concentrated to dryness under reduced pressure and controlled temperature (400 C to 500 C) in a rotary evaporator until all solvent was removed to give a dark colored molten extract. The percentage yield of the methanolic leaf extract of *Nyctanthes arbor-tristis* was *72%*. The extract was stored in airtight containers in refrigerator maintained below 100 C until further use [4, 5].

Synthesis of ZnO NPs

1 mM Zinc acetate was dissolved in 50 ml Milli-Q water and Stirrer for 1 h respectively.20 mL of NaOH solution was slowly added into the Zinc acetate solution 25 mL of plant extract was added .The colour of the reaction mixture was changed after 1 h of incubation time.Solution was left in stirrer for 3 h Yellow colour appeared after the incubation time confirmed the synthesis of ZnO NPs. Precipitate was separated and centrifugation at 8000 rpm at 60 °C for 15 min. pellet was collected and dried hot air oven at 80 °C for 2 h and preserved.

CHARACTERIZATION OF ZNO NANOPARTICLES

UV –Visible Spectroscopy

For UV- Visible spectra of synthesized ZnO nanoparticles were re-suspended in equal amount of sterilized de-ionised water and spectrum scans were performed using Shimadzu UV-1800 Spectrophotometer. 2 ml solution of the nanoparticles was taken in quartz cuvette. The scan range was set between 200-800nm and the background was minimised using de-ionised water.

Fourier Transform Infrared (FT-IR) Spectroscopy

The characterisation of phytoconstituents of *Nyctanthes arbor-tristis* hydro alcoholic extract involved in the reduction and stabilization of zinc nanoparticles was investigated by FT-IR analysis (Shimadzu- IR Affinity-1) and the spectra was scanned in the range of 4000-500 cm^{-1} range at a resolution of 4 cm^{-1}.The sample was prepared by grounding the ZnO nanoparticles uniformly in a matrix of dry KBr, compressed to form an almost transparent disc. KBr was used as a standard to analyse the sample.

Transmission Electron Microscopy (TEM)

TEM technique was used to visualize the morphology of the nanoparticles and determination of the size, shape and arrangement of particles. The ZnO nanoparticles was suspended in sterile deionised water, sonicated for 15 min and diluted to yield slightly turbid suspension. The suspension was then coated onto a copper grid and allowed to dry. TEM images were taken on the Philips CM200 7500 model with resolution 2.4 A0 operating at voltage 20-200kv.

Scanning Electron Microscopy (SEM)

The morphological features of synthesized ZnO nanoparticles from NAT plant extract were studied by Scanning Electron Microscope (JSM-6480 LV). After 24Hrs. of the addition of ZnO the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV.

X Ray Diffraction Analysis (XRD)

The XRD pattern of the synthesized nanoparticles was then recorded using Analytical PW3040/60 XpertPRO model X-ray diffractometer. A thin flim of the sample was made by dipping a glass plate for XRD studies. The instrument was operated at a current of 30mA and voltage of 40KV.The size was calculated using Scherer formula.

= 0.94 λ

β cosθ

Where,

D = crystal size,

λ = wavelength of X-ray,

Θ = Bragg's angle

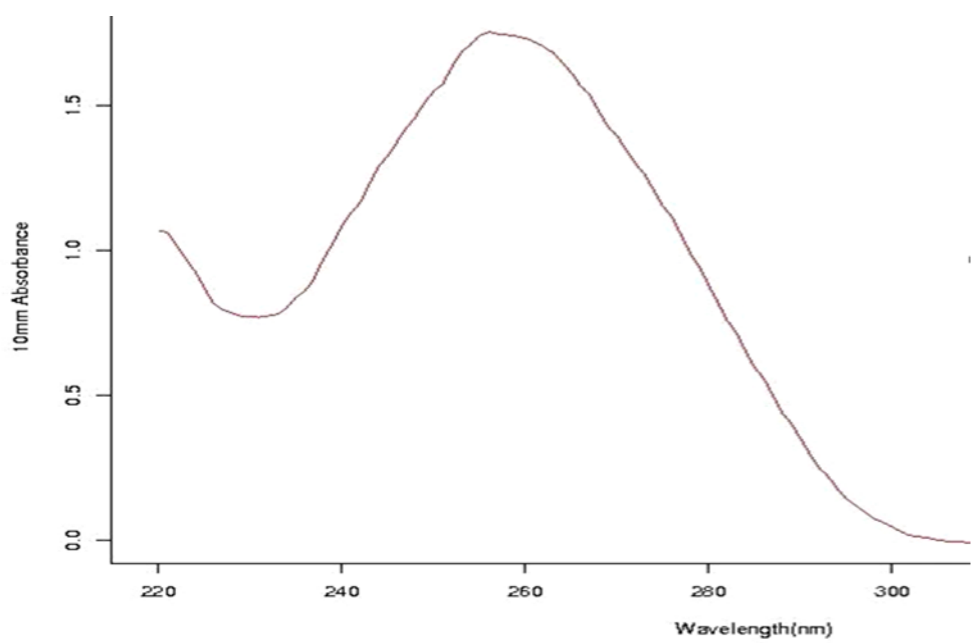
β= Full width at half maxima (FWHM) of spectral peak (in radians)

RESULTS AND DISCUSSION

CHARACTERIZATION OF ZnO NANOPARTICLES

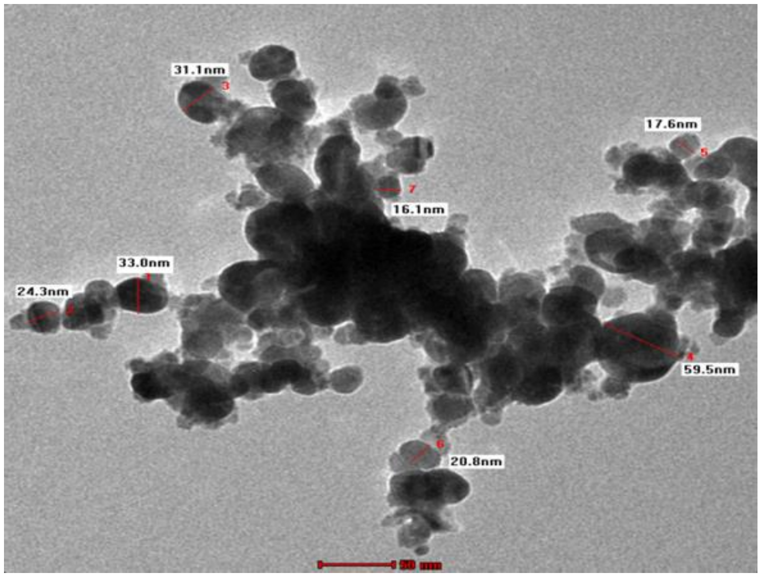
UV –Visible Spectroscopy

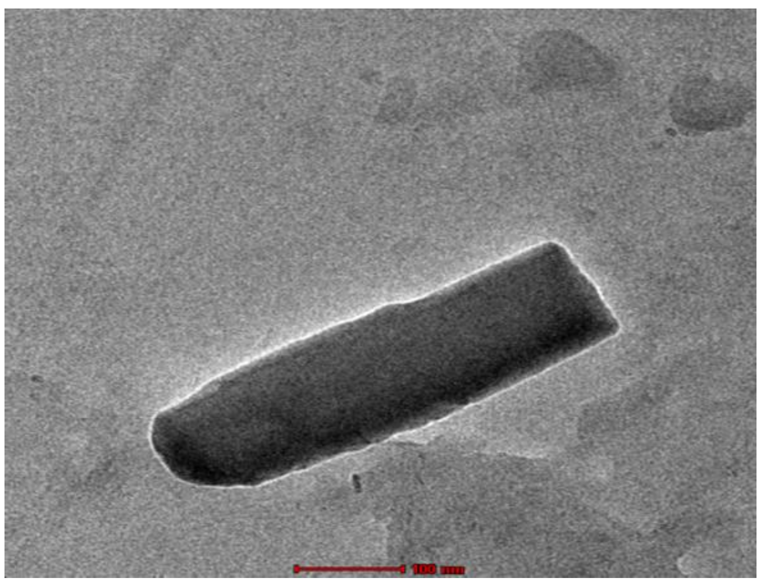
The zinc oxide nanoparticles were efficiently synthesized from *Nyctanthus arbor-tristis* leaf extract with zinc acetate at the ratio of 1:10.With in 30 mins of incubation the dark brown colour changes, an indicator of zinc oxide nanoparticles formation. The green synthesized zinc oxide nanoparticles showed absorbance from 250 to 265 nm and the absorbance centred at 260nm in UV-visible spectroscopy. The UV-visible absorbance spectra result enlightens that the nanoparticles were found to be symmetrical with spherical polydispersed in nature.



Transmission Electron Microscopy (TEM)

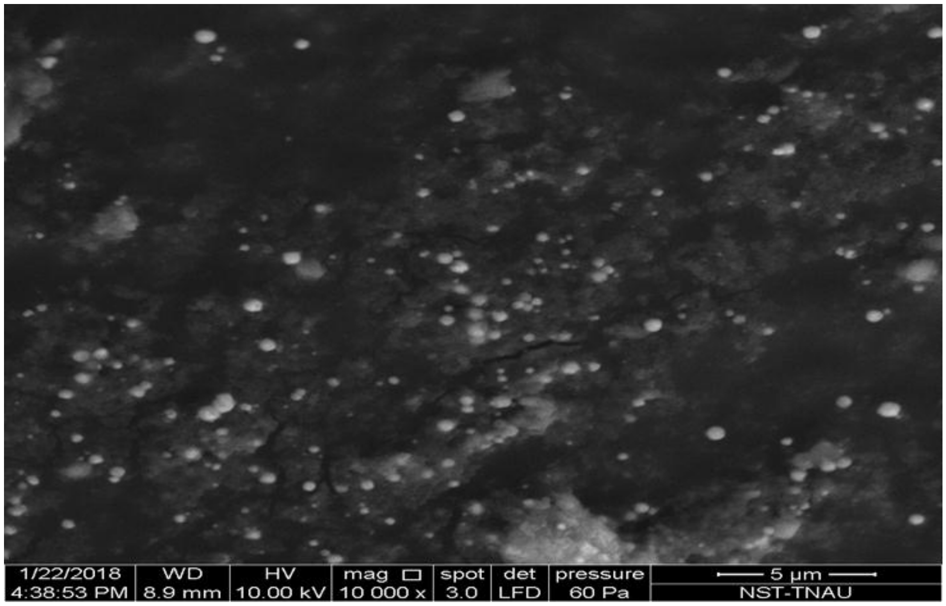
TEM analysis was performed in order to investigate the morphological and distribution of our green synthesized zinc oxide nanoparticles. TEM analysis revealed that all the nanoparticles were found in general as spherical shape. The average particle size measured from the TEM images is found to be 50nm.

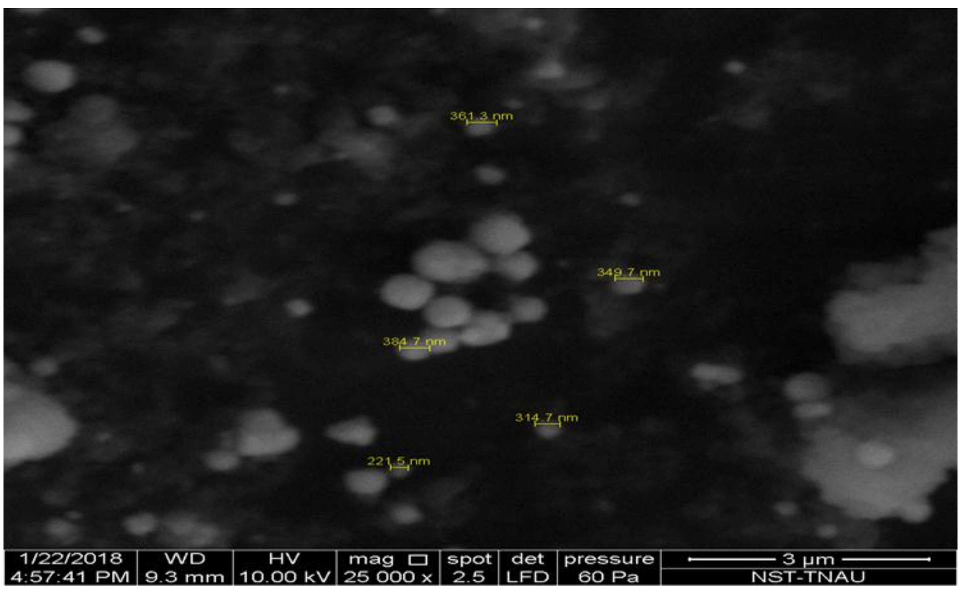




Scanning Electron Microscopy (SEM)

The analysis of the scanning electron microscopy (SEM) images predicts the formation and the morphology of stable zinc oxide nanoparticles obtained from the current green approach. The images showed the presence of both individual as well as the aggregated ZnONPs. The ZnONPs are mainly uniform spherical shaped with the average range of particle size distribution from 40 nm to 80 nm.





X ray Diffraction Analysis

Washed and dried sample of ZnO NPs was used for XRD analysis using Ultima IV at the wavelength of 1.5406 A °. XRD was performed in the 2h range of 20–80 degrees at 40 kV and 40 mA with a divergence slit of 10 mm in 2h/h continuous scanning mode. Crystal lattice indices and particle size calculations were performed using the X-ray diffraction pattern of ZnO NPs. Diffraction peaks were observed at the 2h values of 28.55º, 31.76º, 32.62º,34.42º,36.25º,47.53º,50.66º,56.58º and 62.84º corresponding to lattice planes (70), (86), (90), (113), (114), (145), (200), (208) and (235) respectively.

Interplanar d-spacing was calculated using Bragg's Law equation

$2d \sin\theta = n\lambda$

where,

θ is Bragg's angle of diffraction,

λ is X-ray wavelength, i.e. 1.5406 A ° and n= 1.

Further, particle size was calculated from the intense peak corresponding to (101) plane using Debye–Scherrer formula

D ¼ 0:89k=b cosθ

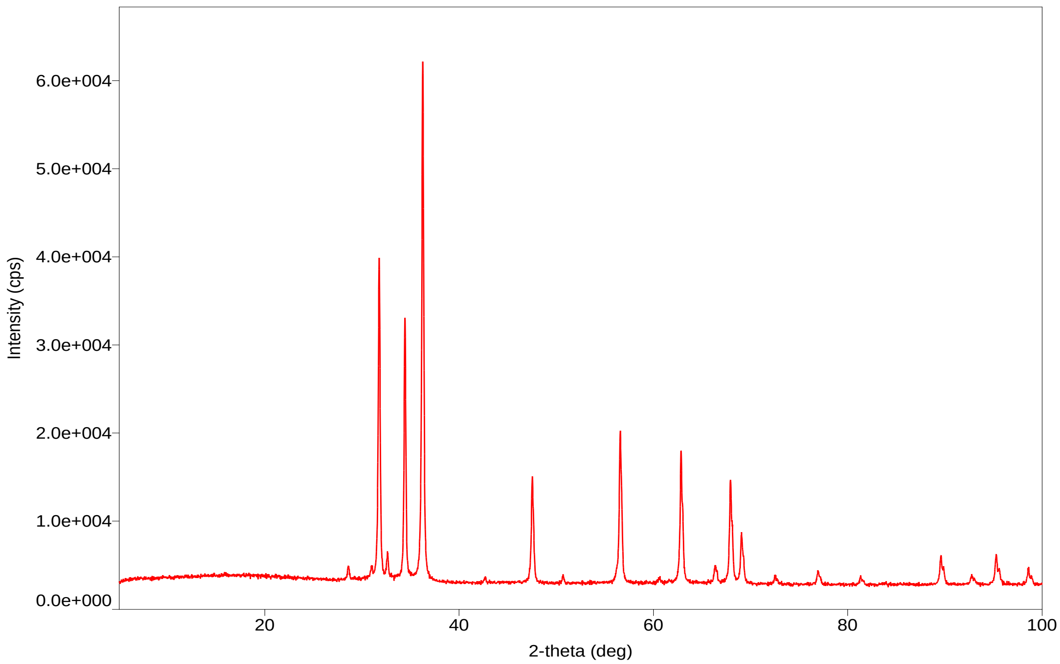
where

0.89= Scherrer's constant,
k= X-ray wavelength

$$= \frac{0.89 \times 1.5406}{0.1061 \times 0.8064}$$

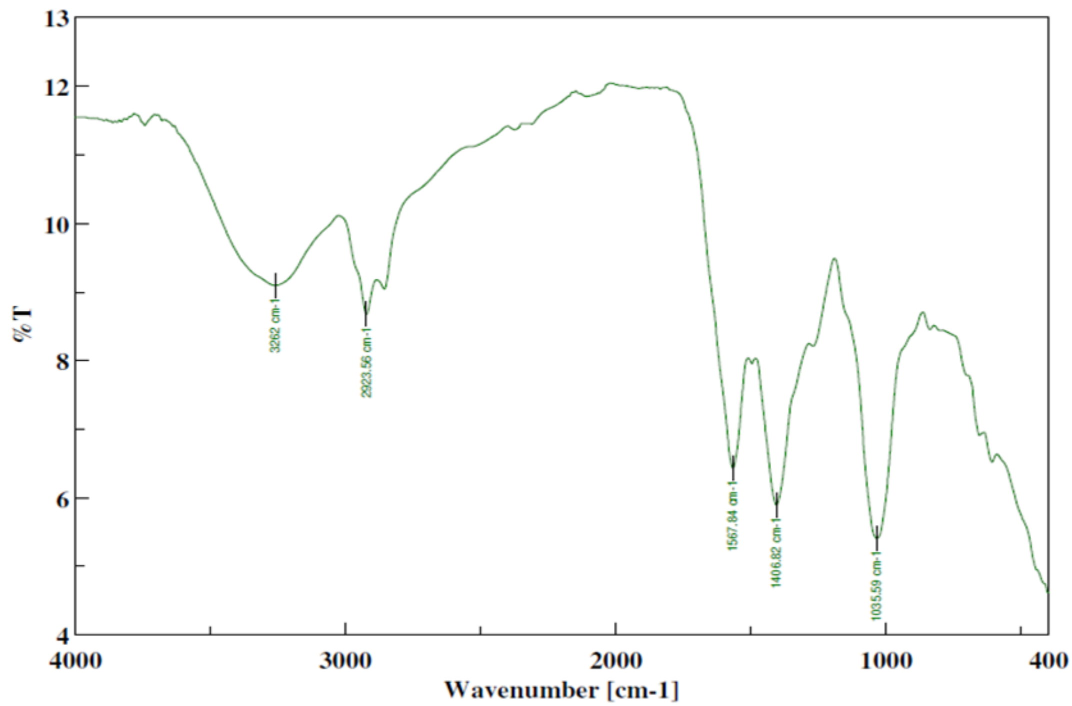
= 16.027

The value of particle size was found to be 16.2 nm which falls within the size range of 15-48 nm reported by TEM and XRD.



Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FT-IR) spectroscopy helps establish the identity of various phyto-chemical constituents involved in the reduction and stabilization of the nanoparticles. FT-IR spectrum for dried and powdered ZnO NPs was obtained using Perkin Elmer FT-IR Spectrophotometer Frontier using the technique of Attenuated Total Reflectance (ATR) in the range of 4000–400 cm. The sample for the infrared analysis was carefully prepared to exclude any possibility of the presence of any unbound plant extract residue. The similarities between the spectra with some marginal shifts in peak position clearly indicate the presence of the residual plant extract in the sample as a capping agent to the ZnO NPs. FT-IR spectroscopy was performed. The FTIR spectra resulted in various peaks at 3262, 2923.56, 1567.84, 1406.82 and 1035.59 cm^{-1}. The peaks at 3262 correspond to H bonded OH stretch and N-H stretch. Peak at 2923.56 corresponds to C-H stretch. The 1567.84 peak refers amine –NH vibration stretch in protein amide. The peak at 1406.82 refers C-C stretch. The 1035.59 peak results from C-O-C stretching in aromatic amine.



In general, metalic nanoparticles are conventionally synthesized by wet chemical methods which require toxic and flammable chemicals. But use of plant extracts (green synthesis approaches) were attained great interest in these days and various metals and metal oxide nanoparticles have been successfully synthesized using plant extract. ZnO nanoparticles are synthesized by various chemical methods like vapor transport, hydrothermal synthesis, precipitation method etc. So far, various parts of plants were used to synthesize ZnO nanoparticle with assured biological activities. Leaf extracts of *Camellia sinensis*, gel of *Aloe barbadensis*, flower extract of *Nyctanthes arbor-tristis*, stem bark of *Boswellia ovalifoliolata*, seeds of *Trachyspermum ammi* and latex of *Calotropis procera*. The present study utilized and states that green approach for the synthesis of zinc oxide nanoparticles employing methanolic leaves extract of Nyctanthes arbor-tristis.

CONCLUSION

In the present study on ''Green synthesis of zinc oxide nanoparticles using leaves extract of Nyctanthes arbor-tristis and their antifungal activity", zinc oxide nanoparticles were synthesized using methanolic leaves extract of Nyctanthes arbor-tristis. Synthesis conditions were optimized and resultant nanopowder was characterized using UV- Visible spectroscopy, XRD, FT-IR, SEM and TEM. TEM images report individual particle size range of 12–32 nm and also revealed that the nanoparticles are present in the form of aggregates. In addition to morphological analysis, TEM analysis also establishes the role of a lesser intense capping layer on the NP surface. It is simple, cost-effective, rapid and eco-friendly way to synthesize zinc oxide nanoparticles and capable of synthesizing within few hours at room temperature. The use of plant extracts avoids the usage of harmful and toxic reducing and stabilizing agents. Zinc nanoparticles can exist in ions only in the presence of strong oxidizing substances. The environmental conditions will affect the stability of nano particle. The synthesized nanoparticles may be further explored for preparation of nano medicines and other products.

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