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GREEN SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND FOOD PACKAGING APPLICATION OF BIOCOMPATIBLE ZINC OXIDE NANOPARTICLES

P. Ramesh*[1] and K. Saravanan[2]

[1*]Department of Physics, Nehru Memorial College (Autonomous), Puthanampatti, Tiruchirappalli, Tamilnadu - 621007, India.

[2]Department of Zoology, Nehru Memorial College (Autonomous), Puthanampatti, Tiruchirappalli, Tamilnadu- 621007, India.

ABSTRACT

The present study described the synthesis of biocompatible Zinc oxide nanoparticles from the zinc acetate through eco-friendly green process using leaf extract of *Phyllanthusniruri* leaf and its antibacterial activity and its application in food packaging. The green synthesized Zno nanoparticles were characterized by Fourier-transform infrared (FTIR) spectroscopy, Ultra violet spectroscopy, Photoluminescence spectroscopy, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM). FTIR studies confirm the presence of biomolecules and metal oxides. UV spectrum and PL spectrum study revealed the optical properties of ZnO NPs. X-ray diffraction (XRD) structural analysis reveals the formation of a high rate of crystalline behaviour and controlled morphology. The surface morphologies of ZnO nanoparticles observed under a Fourier-transform scanning electron microscope (FE-SEM) suggest that the presence of Zine and Oxygen molecules on the surface area and the most ZnO crystallites are spherical in shape. Then it was tested against four bacterial pathogens viz., *Bacillus subtilis*, *Klebsiella pneumo*nia, *Pseudomonas aeruginosa* and *Proteus mirabilis*. The biosynthesized nanoparticle synthesized from leaf extract of *cassia auriculata* exhibited strong antibacterial activity against *Proteus mirabilis and Pseudomonas aeruginosa.* Since ZnO nanoparticle synthesized from *Phyllanthusniruri* leaf has potent antimicrobial activity, it may be incorporated in polymeric matrices in order to provide antimicrobial activity to the packaging material and improve packaging properties.

KEYWORDS

Green synthesis, ZnO nanoparticle, *Phyllanthusniruri*, Antibacterial activity and Food packaging.

Author for Correspondence:

Ramesh P,

Department of Physics,

Nehru Memorial College (Autonomous),

Puthanampatti, Tiruchirappalli, Tamilnadu, India.

Email: p.ramesh704@gmail.com

INTRODUCTION

Nanotechnolgy is the art and science of manipulating matter of the nanoscale to create new and unique materials and produces with enormous potential to change society (Kavitha *et al*, 2013[1], Krause, 2014)[2]. Nanoparticles have a wide range of application like electronic, magnetic, optical, cosmetics, sunscreen lotions, biomolecule detection, diagnostics and drugs (Singhal *et al*, 1997[3], Schilling *et al*, 2010[4], Poinern *et al*, 2015)[5]. The inorganic NPs such as silver, gold, copper, CuO, TiO2 and ZnO have profound antibacterial activities. Among the inorganic NPs, ZnO NPs are of particular interest because they can be prepared easily inexpensive and safe material for human welfare. They are extensively used in the formulation of health care products (Manna *et al*, 2013)[6]. ZnO NPs have entered the scientific spotlight for its semiconducting properties, wide range of biological activities, UV filtering properties, high catalytic and photochemical activity (Nagajyothi *et al*, 2013)[7]. Zinc oxide is a semiconductor with wide band gap (3.37), high excitation binding energy (60 meV) at room temperature (Roduner, 2006[8], Jones *et al*, 2011)[9] and has unique optical and as well as excellent thermal and chemical stability (Iravani, 2013)[10]. ZnONps is synthesized by chemical, physical and biological methods. Plant extracts mediated synthesis of nanoparticles has been proved advantageous over physical and chemical methods (Makarov *et al*, 2014)[11]. Use of plant extracts as natural reducing, capping and stabilizing agents have been attained progress and are reported by many authors as green synthesis method an efficient one (Shekhawat *et al*, 2014[12], Hussain *et al*, 2016)[13]. The biological activity of ZnO nanoparticles with minimal toxicity led to its use in many biomedical and food packaging applications (Hossain *et al*, 2013)[14]. In the present study, the leaf extract of *Phyllanthusniruri* leaves were used for the synthesis ZnO nanoparticle. *P.niruri* leaves havea wide range of medicinal activities including antihepatotoxicity, antidiabetic and antimicrobial activities (Obiagwu *et al*, 2011[15], Makarov *et al*, 2014[11], Senthilkumar *et al*, 2014)[16]. Plants are rich in pytochemicalsparticularly secondary metabolites such as tannins, terpenoids, flavonoids that have been demonstrated to have antibacterial properties. *P.niruri* was reported to possess alkaloids, tannins, and flavonoids by Kaur Ramandeep *et al* (2017)[17]. Thus in the present study, it is hypothesized that the use of leaf extract of *Phyllanthusniruri* leaves as caping agent would increase antibacterial activity of ZnO nanoparticle and also the incorporation in to the food packaging materials prevent the entry of microbes.

MATERIAL AND METHODS

Plant material

The leaves of *P. niruri* were collected from Puthanampatti, Thuraiyur, Tiruchirappalli, Tamilnadu (Lat. Long) and brought to the laboratory. They were washed thoroughly and allowed to dry at room temperature. The plant was authenticated by Dr. M. Meenakshi Sundaram, Head and Assistant Professor of Botany, Nehru Memorial College, Tiruchirappalli.

Preparation of the leaf extract

Dried leaves of *P. niruri* were finely powdered with domestic grinder. One gram of powder was boiling with10 ml of deionized water at 100°C for 10 minutes and filtered through a Whatmann No.1 filter paper. Then the filtrate was stored at 4°C for the ZnONps preparation.

Preparation of zinc nanoparticles

In this method, 3.3g of Zinc acetate (0.1 M) was dissolved in 100ml of double distilled water. It was thoroughly mixed under continuous stirring process for 10 min at room temperature. Then it was titrated with 5 ml of P. *niruri* leaf extract for 2 hours under continuous stirring process. The pH value of solution was maintained at 12. After 2hrs the solution was centrifuged at 6000 RPM for 30mins. The mixture was then boiled for 60 minutes until the aqueous solution changes from watery to light green paste. This paste was then collected in a ceramic crucible and heated in an air heated furnace at 400 degree celsius for 2 hours. A light white coloured powder was obtained and this was carefully collected and packed for characterization. The material was mashed in a mortar pestle so as to get a finer nature for characterization.

Characterization of green synthesized ZnO nanoparticles

UV- Vis Analysis

UV – Vis absorbance spectrum of the sample was recorded by UV – Vis spectrometer (M/S JASCO, Model V-670, and U.S.A). The Photodegration of crystal violet dye was measured by UV-Vis Spectrometer (M/s Shimadzu, Japan).

XRD analysis

From the XRD measurement, crystalline phase and crystallite size of ZNO NP where characterized using powder X-ray diffractometer (M/S PANalyticalX'Pert Pro, The Netherlands), operated at voltage= 40KV and current = 30m A with Cu-Kα (λ = 1.5406 Å) radiation.

FT-IR Analysis

Functional group of the materials was confirmed by Fourier Transformation Infrared spectrometer (M/S JASCO, U.S.A) in the transmittance mode in the range of 4000-400 cm^{-1} using KBr pellet.

SEM And EDX Analysis

The surface morphology of as synthesized product was analysed by scanning electron microscopy (ZEISS). The energy dispersive X-ray Spectrometer was performed by a (ZEISS) EDX Spectrometer to determine the elemental composition of the sample.

Antibacterial Assay

The following bacterial strains were used in this study viz., *Bacillus thuringiensis* (ATCC 10792), *Escherichia coli* (ATCC 25922), *Staphylococcusaureus* (ATCC 6538) and *Pseudomonasaeuroginosa* (ATCC 15442). Disc diffusion method was carried out by using standard protocol and overnight bacterial cultures (100mL) was spread over Muller Hinton Agar (Hi Media Laboratories Private Limited, Mumbai, India) plates with a sterile glass L-rod. 100mL of the each extracts were applied to each filter paper disc Whatmann No.1 (5 mm dia) and allowed to dry before being placed on the agar. Each extract was tested to triplicate and the plates were inoculated at 70Cfor 24 hours after incubation. The diameter of inhibition zones was measured and tabulated.

RESULTS AND DISCUSSION

UV-Vis Spectroscopy analysis green synthesized ZnO NPs

Optical properties of the as-prepared ZnO nanostructure sample was revealed by UV–Vis spectroscopy at room temperature, as shown in Figure No.1. It can be seen from the Figure that there was intensive absorption in the ultraviolet band of about 300-1100 nm. The absorption wavelength at about 369 nm of ZnO suggested the excitonic character at room temperature.

The determination of optical band gap is obtained by Tauc's equation (Bhatt *et al*, 2012)[18],

$$\alpha h\nu = A\,(h\nu - E_g)^n$$

Where, A is a constant, hν is photon energy, E_g is the allowed energy gap, n = ½ for allowed direct transition and n = 2 for allowed indirect transition. Figure No.1(a) shows the absorption coefficient (α) and the two defined regions separated by the peak of derivative of absorption coefficient. The Tauc's region is extrapolated to $(\alpha h\upsilon)^2=0$. To obtain the band gap. The Urbach's region tells the structural and thermal disorder present in the film. Figure 1(b) shows the $(\alpha h\nu)^{1/2}$ vs. hν for Zno nanoparticle.

$$E_g=\frac{hc}{\lambda}ev;\ E_g=\frac{1240}{\lambda}ev$$

The band gap energy corresponds to the absorption limit and can beroughly evaluated by the above relation. Where, Eg is the band gapenergy (eV), h is the Planck's constant ($6.626\ X10^{-34}$J s), C is the light velocity ($3X10^8$ m/s) and k is the wavelength (nm). From figure. The absorption edge are positioned at 369 nm which, corresponding the band gap value of 3.33 eV which indicates red shift in the UV region.

Photoluminescence (PL) analysis

Photoluminescence (PL) studies were performed to emphasize its emission properties as shown in Figure No.2. The photoluminescence of ZnO sample suggested four emission bands at 391nm, 411nm, 438nm and 491nm. The blue band at 411nm, 438nm correlation with the defect structures in ZnO crystal. The Blue-green band 491nm may be in correlated to a transition between the oxygen vacancy and interstitial oxygen (Vanheusden *et al*, 1996[19], Shim *et al*, 2003)[20].

XRD pattern of green synthesized ZnO NPs

The X-ray diffraction patterns of the synthesized ZnO are shown Figure. The experimental diffraction pattern of ZnONPs as certain 2θ peaks values are at: 31.76°, 34.42°, 36.24°, 47.51°, 56.57°, 62.80°, 66.26°, 69.15°, 72.43°and 76.99°and can be attributed to the Miller planes of (100), (002),(101), (102),(110), (103),(200), (201), (004) and (202) which agrees with the anatase phase structure and diffraction patterns were given as shown figure, (Figure). From figure it can be inferred, the intensity of peaks for sample indicated a higher rate of crystalline behaviour and controlled morphology. The peaks have been attributed to hexagonal phase of ZnO (JCPDS file: 36-1451) ((Khoshhesab *et al*, 2011[21], Talam *et al*, 2012[22], Zhou *et al*, 2007)[23].

As the width of the peak as increased as the particle size decreased. Their crystallites size 24 nm. It was notice that averages crystallites size of nanoparticles were smaller than stander nanoparticles averages 79.5 nm.

FTIR analysis of green synthesized ZnONps

FTIR Spectra of aqueous Zinc oxide nanoparticles prepared from the Phyllanthus Niruri leaf extract was carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by leaf broth. The finding of Figure, showed fundamental mode of vibration at 649 cm-1, and 1412 cm-1 for standard NPs which correspond to the Zn-O stretching vibration. The Zn-O stretching vibration of biosynthetic NPs observed at: 649 cm-1, 586 cm-1, 1412 cm-1 and 1541 cm-1. For all of these peaks correspond to the Zn-O stretching vibration according to NIST WEB BOOK, (http://webbook.nist.gov/).

SEM and EDAX analysis

SEM studies were revealed to visualize the size and shape of Zinc oxide nanoparticles and (Figure No.5) and show the typical bright –field SEM micrograph of the synthesized Zinc oxide nanoparticles. In this study, it was appeared with that most of spherical in shape. The synthesized ZnO NPs were subjected to an EDAX spectrum to quantify the mixture of metal and oxides present in the sample. The results showed that 62.79% of Zn and 37.21% of O were present on the surface area, as clearly shown in Figure No.5.

Antibacterial activity of green synthesized Nps

The mean zone of inhibition of growth of different bacteria is given in Table (5). Green synthesized ZnO nanoparticles 400°C annealed at 200mg/ml concentration exhibited high antibacterial activity against all test bacteria under study. The highest zone of inhibition (20.5±1.32) was observed against *P. mirabilis* followed by *K. Pneum*onia (18.2±1.76) (Figure No.5). The present study revealed that the green synthesized zinc oxide nps have potent antibacterial activity against bacterial pathogens *viz., B. subtilis*, *K. pneum*onia *P. mirabilis* and *P. aeruginosa.* Among these, *P. mirabilis* and *P. aeruginosa* are more susceptible to the green synthesized ZnOnps. The antimicrobial efficacy of green synthesized ZnONps may be due to the action of *P. niruri* leaf extract act as capping agent in Nps and to reduce the size of particles and enhances the antimicrobial activities (Shubhangi *et al*, 2014)[24]. Nanoparticles size decrease in antimicrobial activity increases, this is due to higher surface to volume ratio and more bioactive compounds of the plant extract available, so smaller nanoparticles penetrate easily within the bacterial cell membrane and possess higher bactericidal effect (Bala N. *et al*, 2015)[25]. From the XRD patterns, it is confirmed that the crystallite size of green synthesized ZnO NPs are found to be 24 nm and 30 nm for ZnO 400°C annealed.

Since ZnONps have shown antimicrobial properties they may have potential applications in food preservation. Thus it is concluded that ZnO nanoparticles may be incorporated in polymeric matrices in order to provide antimicrobial activity to the packaging material and improve packaging properties.

S.No	Element	Weight%	Atomic%
1	C K	20.06	35.92
2	O K	37.21	50.02
3	Zn K	42.74	14.06
4	Totals	100.00	100.00

Table No.1: Antibacterial activity of green synthesized ZnOnps

S.No	Species	Mean zone of inhibition (mm)			
		Green synthesized ZnO nanoparticles 400° C annealed at different concentration			
		50mg/ml	100mg/ml	200mg/ml	Antibiotic
1	***Bacillus subtilis***	5.0±0.41	6. 1±0.94	17.4±0.79	10.1±1.11
2	***Klebsiella pneumo*nia**	10.2±1.10	10.9±1.40	18.2±1.76	15.1±0.87
3	***Pseudomonas aeruginosa***	4.5±1.27	8.3±0.93	9.2±1.26	12.0±1.53
4	***Proteus mirabilis***	14.2±0.76	10.3±1.42	20.5±1.32	15.0±1.35

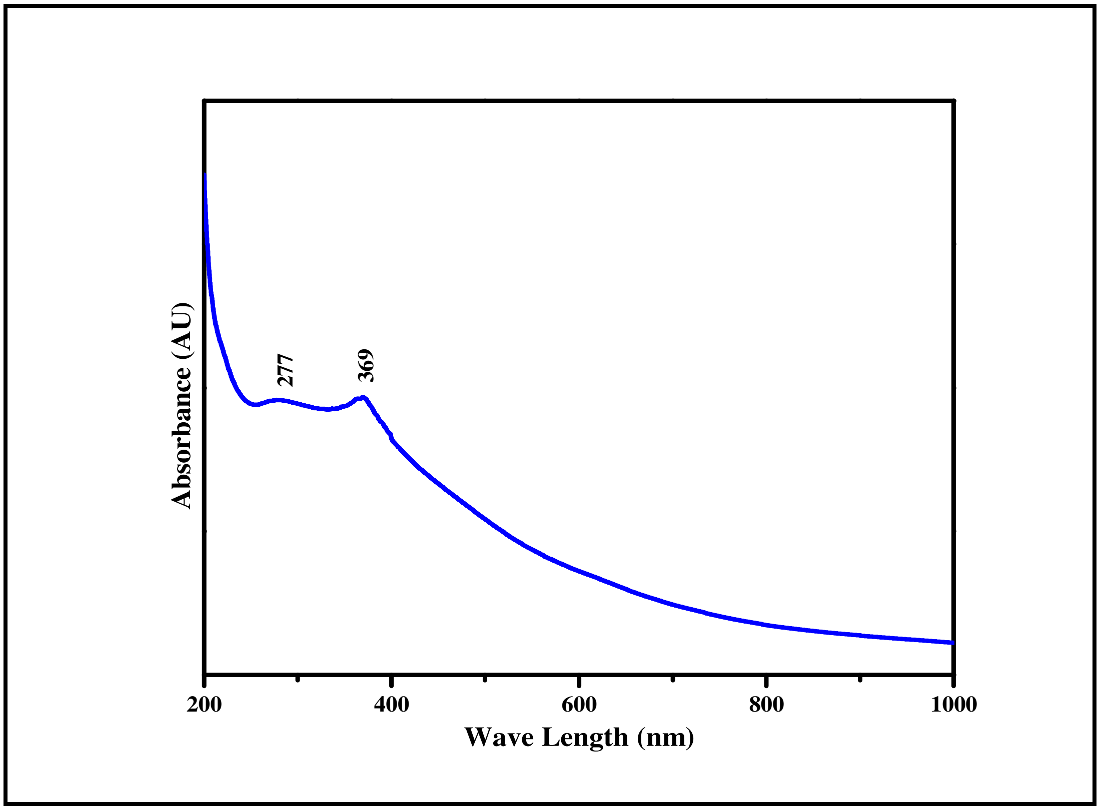

Figure No.1A: UV-Vis spectra of ZnONps

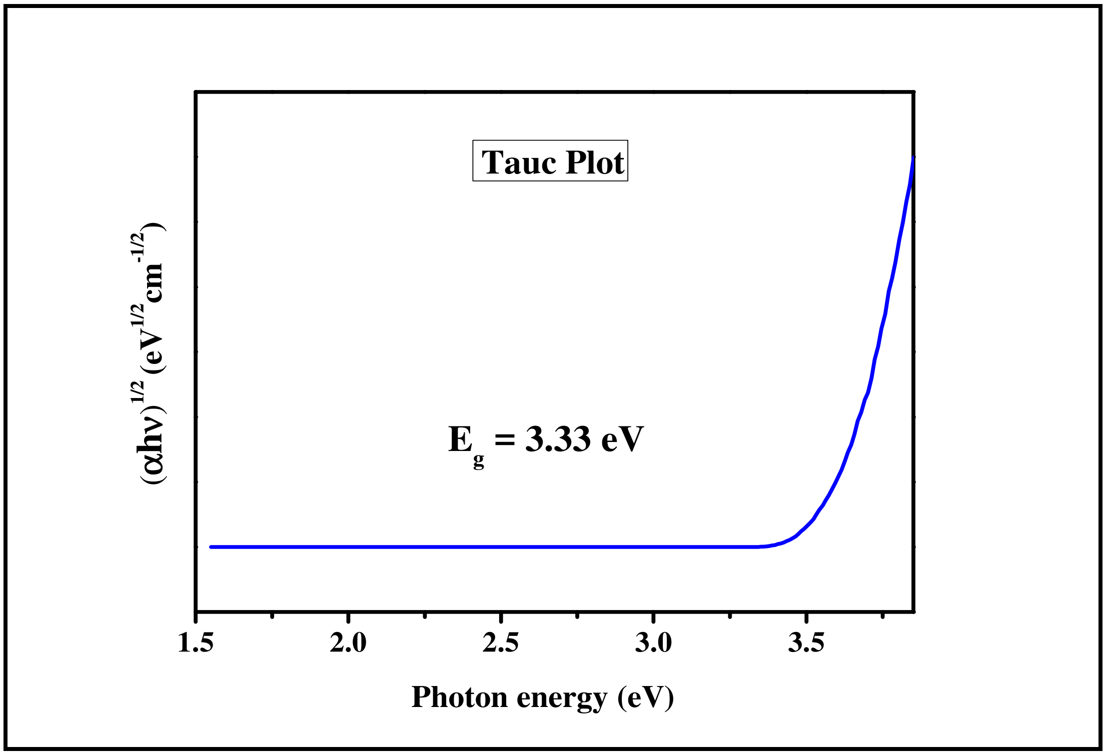

Figure No.1B: Tauc's plot graph of band gap of ZnONps

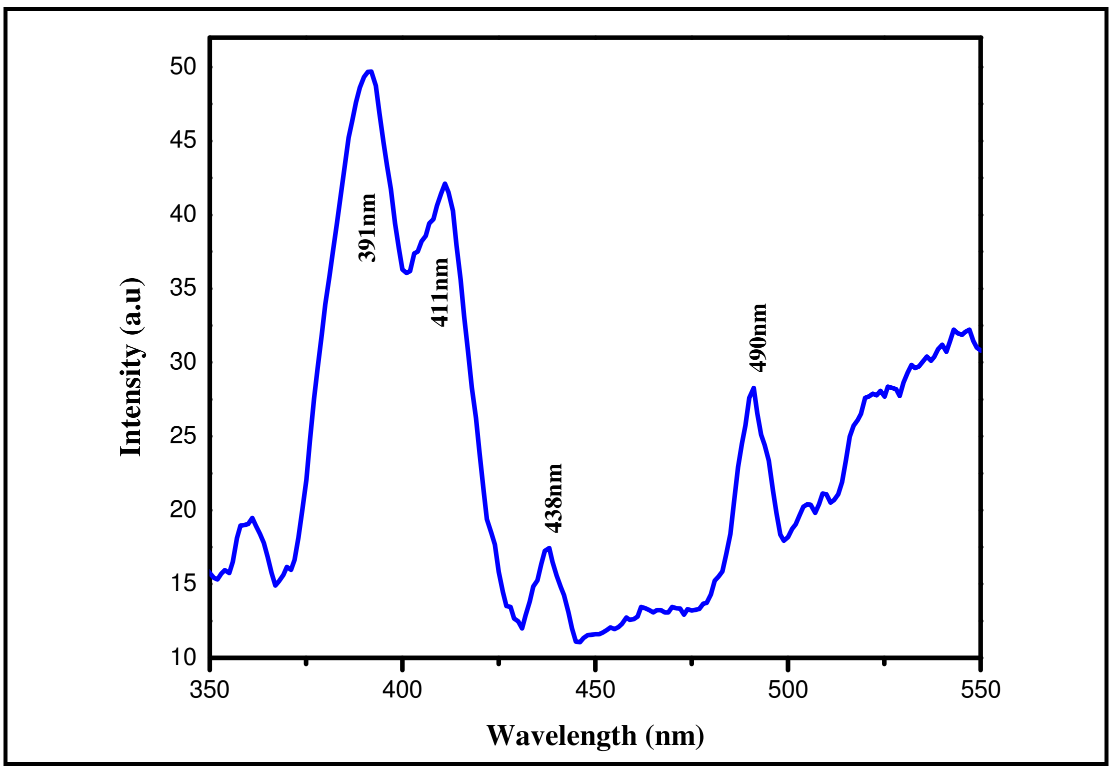

Figure No.2: PL spectra of ZnONps

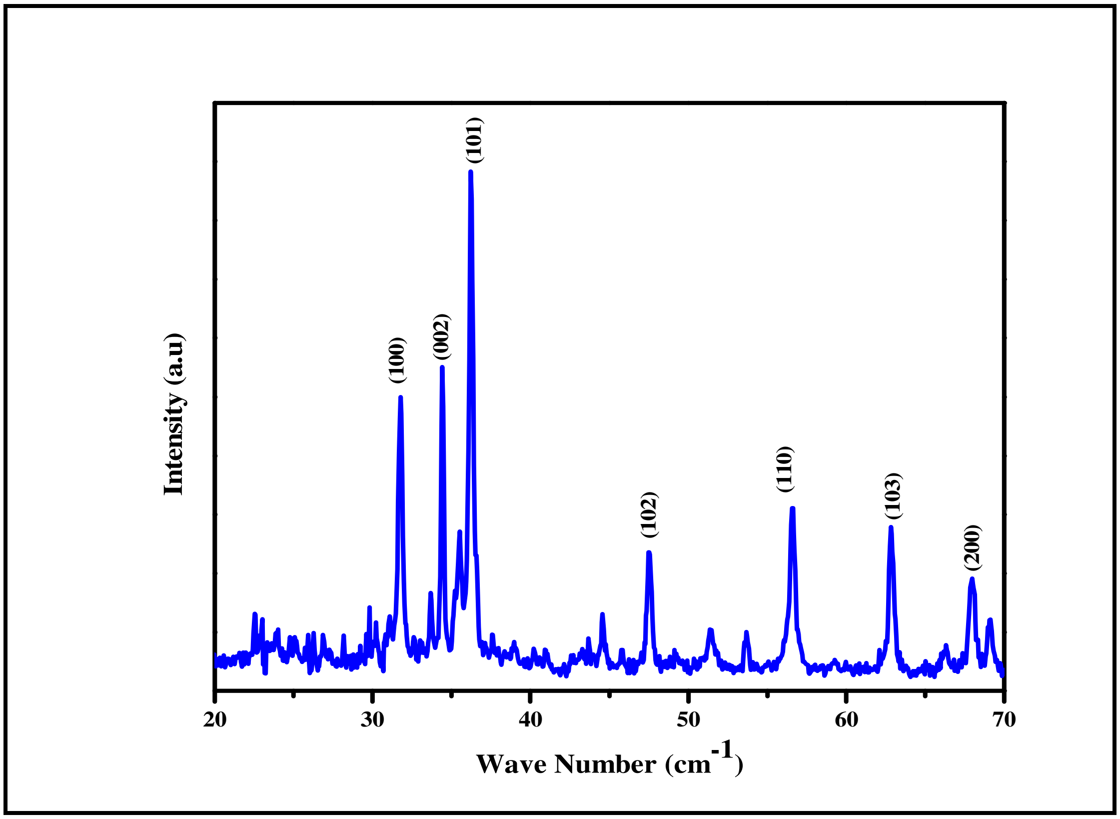

Figure No.3: XRD pattern of green synthesized ZnONps

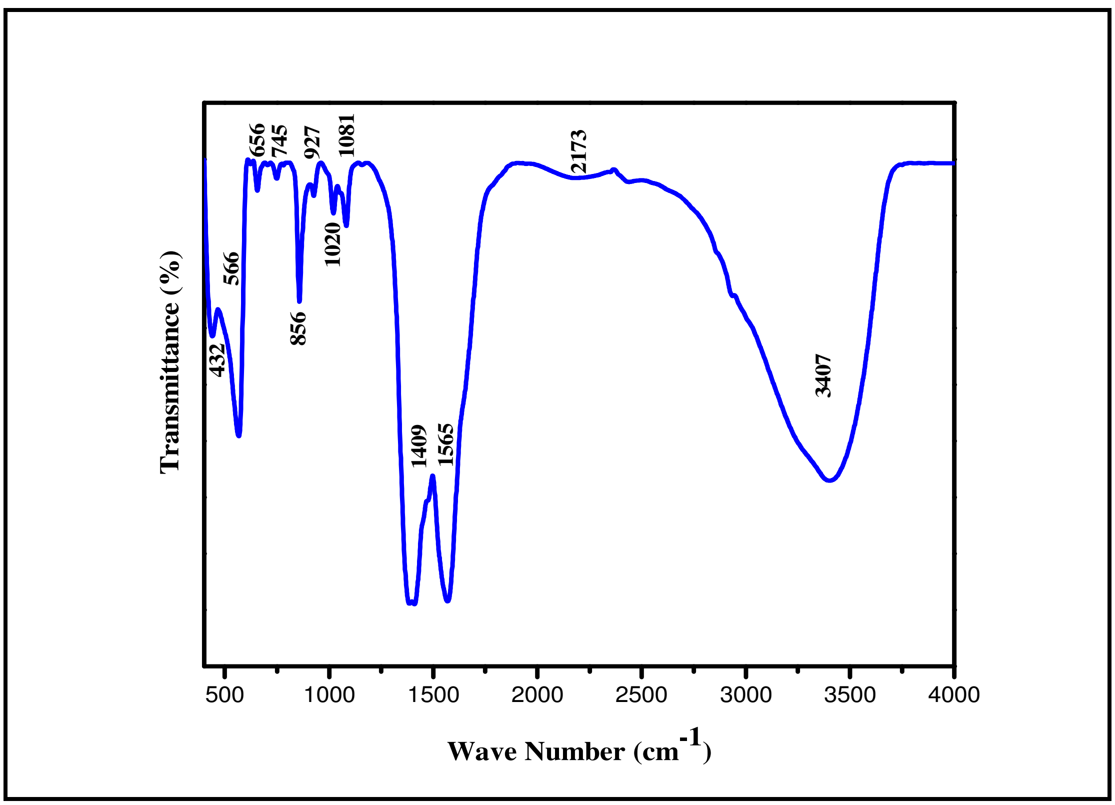

Figure No.4: FT-IR Spectra of green synthesized ZnONps

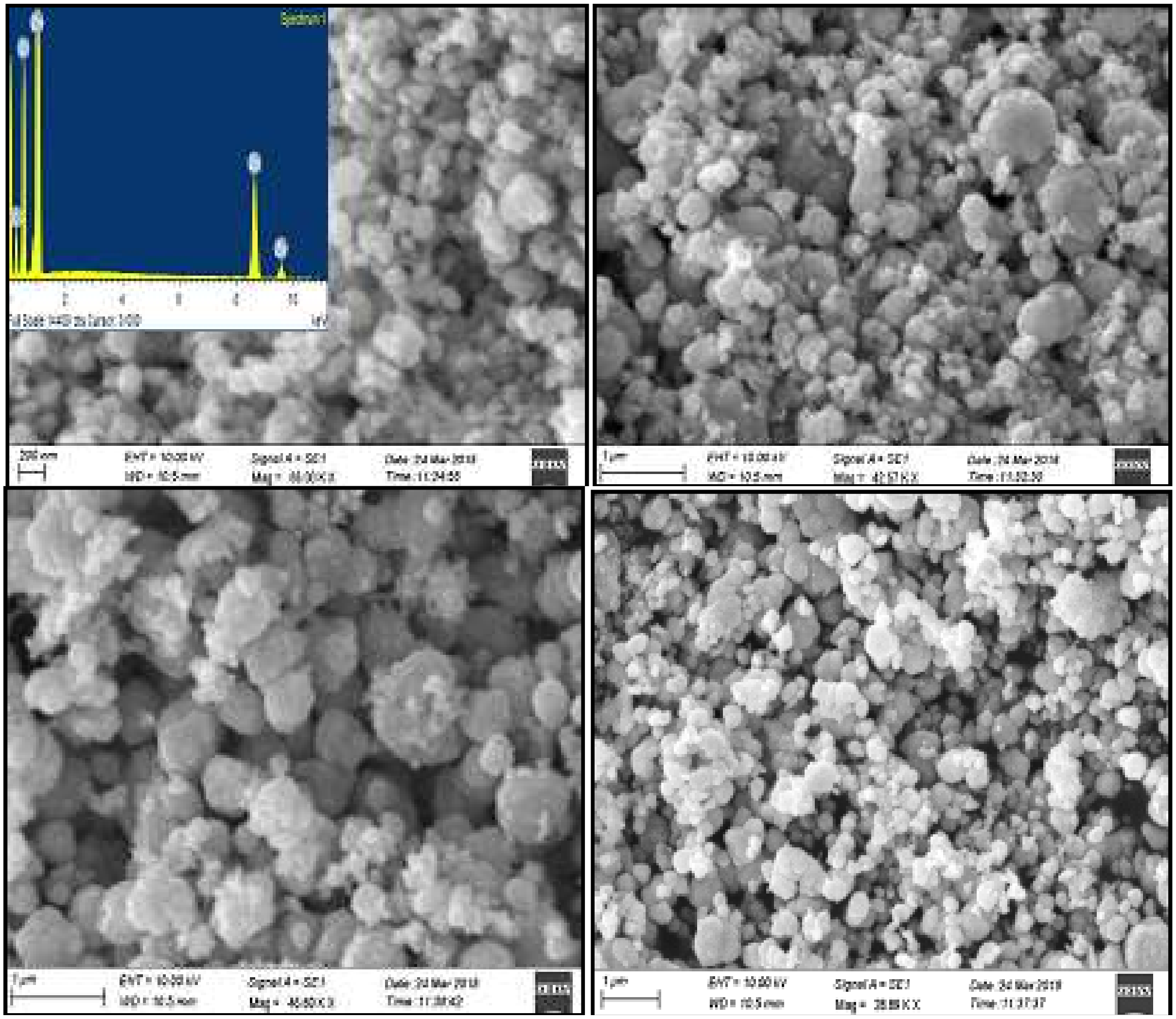

Figure No.5: SEM Analysis and EDX of green synthesized ZnONps

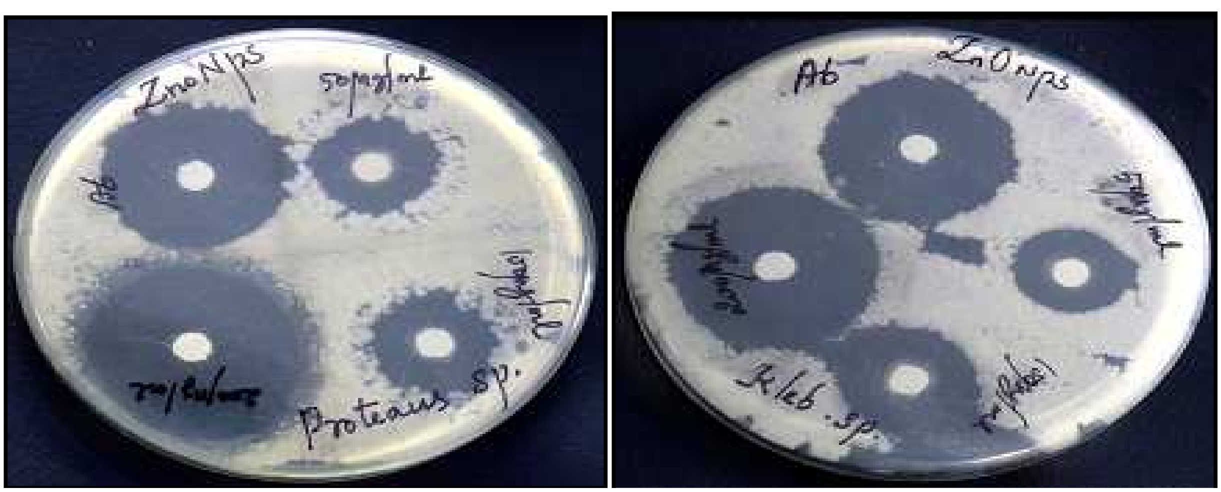

Figure No.6: Antibacterial Activity of green synthesized ZnONps

CONCLUSION

Our findings could be targeted for the promising potential applications including because of its pollution free and eco-friendly approach. This green synthesis approach shows that the environmentally benign and renewable leaf extract of *Phyllanthus Niruri* leaf be used as an effective stabilizing as well as reducing agent for the synthesis of zinc oxide nanoparticles. The antibacterial activity of green synthesized ZnONps showed high antibacterial activity. Thus, the eco-friendly and high efficient zinc oxide nanoparticles synthesized using *Phyllanthus Niruri* leaf extract are expected to have more extensive applications in biomedical filed. Further, packaging films with green synthesized ZnO nanoparticles may be used for food packaging after studying its hazardous effect.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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