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Green Synthesis, Characterization And Antioxidant Activity Of ZnO Nanoparticles Mediated By Aerial Parts *Of Leucas lanata*

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Abstract: The hills of Uttarakhand have been known for medicinal plants since Vedic period. *Leucas lanata* (Dhurlu grass) is also found in this region which belongs to the *Lamiaceae* family was explored for the green synthesis of ZnO nanoparticles . The aqueous extract of arial part of *Leucas lanata* is used for reduction of Zn^{+2} of 20mM of hydrated zinc acetate solution. UV, FTIR, FESEM, EDX and XRD technique are used for the characterization of ZnO nanoparticles. UV-VIS absorption peak observed at 350 and 397nm are due to surface plasma resonance. EDX analysis showing the presence of phytochemicals. The FTIR absorption peaks at 3411$cm^{-1,}$ 2925cm^{-1},1618 cm^{-1},1499 cm^{-1},1410 $cm^{-1,}$1268 $cm^{-1,}$1048 $cm^{-1,}$796 cm^{-1} and 610 cm^{-1} are observed indicating the presence of biomolecules as capping reagent. The spherical morphology with particles sizes revealed byXRD, TEM and FESEM analysis. XRD pattern showing crystalline cubic structure of ZnONPs. Antioxidant activity observed by DPPH assay using ascorbic acid as standard.

Keywords: Vedic • arial • FTIR • morphology • capping • antioxidant activity • DPPH

Introduction

Nanoscience's explore various methodologies, for the synthesis of metallic nanoparticles with unique size and shape due to their wide applications and diversified uses. Metal like Ag, Zn, Pt, Au, Cu, Mg, Pd, etc., are generally used for the synthesis of metallic nanoparticles, among which nanoparticles of metals viz. gold, silver, platinum and zinc are widely applied in fast-selling consumer goods such as shampoos, shops, detergents, shoes, cosmetic products, and toothpastes besides their applications in medical and pharmaceuticals products (Padalia 2015; Mittal 2012; Hemmati 2019; Kumar 2021; Adelere2020) Many metallic nanoparticles show promising results in medical applications i.e. anti-bacterial, anti-cancer, and antioxidant activity, and therefore can play a major role in saving human life in the future (Adelere 2020; Lanje 2010; Parveen 2016). Due to the excessive use of metallic nanoparticles in various fields, researchers are making metallic nanoparticles through a process that is completely green chemistry-based and economically good so that human needs can be met at a low cost without harming the environment (Parveen 2016; Mahmoodi 2018), Two main methods of making metallic nanoparticles are (a) top to bottom method and (b) bottom to top method (Bao 2021; Jamkhande 2019; Abid 2022; Kumar H 2018). In the first method, nano-size metallic particles are made from large metal pieces, for which physical methods like mechanical milling, laser ablation and sputtering etc., are commonly used (Jamkhande 2019; Salah 2011; Gondal 2009; Thareja 2007; Nohavica 2010) and are time-consuming and expensive due to high electricity consumption(Agarwal 2017). On the contrary, in the second method, metallic nanoparticles are synthesized by coprecipitation (Manzoor 2009; Devi 2016) pyrolysis (Ghaffarian 2011), microemulsion (Kumar 2013; Yıldırım 2010), sol-gel method (Hasnidawani 2016), vapor deposition (Liu 2004)etc., which consumes harmful and expensive chemicals (Jamkhande 2019).Therefore nowadays manufacturing of metallic nanoparticles using organic material is full of curiosity for the researcher (Pantidos 2014; Thakkar K 2010) due to its economical and eco-friendly nature.

Several plants have been reported in previous work for the synthesis of ZnO nanoparticle like *Cinnamon camphora*(Zhu 2021), *Salvia officials* (AlrajhiA 2021),*Deverratortuosa*(Selim 2020), *Laurus nobilis*(Fakhari 2019), *Aloe barbadensis miller* (Sangeetha 2011), *Atalantiamonophylla*(Vijayakumar 2018), *Hibiscus subdariffa* (Bala 2015) etc.

Leucas lanata, commonly known as wooly leucasis a softly densely wooly-haired perennial found in the Himalayas mountains range and in south India at 700-to-300-meter altitude.It belongs to*Lamiaceae* family and is indigenously known as Biskapra or Gumma (Dixit 2015; Pala 2011). Locals in Uttarakhand utilize the extract of the arial portion of the *Leucas lanata* plant to treat head and stomach aches, pertussis, and other ailments (Pala 2011;Vermaa 2020). Local tribals of Uttarakhand uses its leaves and flowers with cold water or milk to cure diseases such as cold, cough, and dysentery, while its paste is used to heal wounds (Dixit 2015). HPLC analysis reveals the presence of polyphenols in the *Leucas lanata* plant, including gallic acid, caffeic acid, chlorogenic acid, ferulic acid, and protocatechuic acid (Joshi 2014), which can help in reducing Zn^{++} ion to form Zno nanoparticles. Given the significance of leucas in medicine, the goal of the current work was to create, characterize, and test the biological activity of zinc oxide nanoparticles made from leaves extracted for use in the healthcare sector (Khalafi 2019; Hou 2018; Meulenkamp 1988).

Materials and Methods

Chemicals: The used precursor Zinc acetate and NaOH salt was purchased from Hi Media laboratories Pvt. Ltd. and Nice Chemical Pvt. Ltd. respectively. The glass materials used are sterilized and are of borosil grade.

Apparatus: Magnetic stirrer with hot plate, Digital laboratory centrifuge machine (Navyug India Company)

Collection of plants materials: *Leucas lanata* used in the present study was collected from Karanprayag, (Chamoli) Uttarakhand and was identified by Botanical survey of India Dehradun., where a voucher specimen was deposited.

Preparation of the extract.: Fresh arial part of*Lucas Lanata* was carefully washed with tap water 2 - 3 times and then with distilled water to remove all the dust and visible particles on the surface of pant. Dried in the shade at room temperature(Jayappa 2020). Thereafter, the dried plants were crushed into a fine powder. 20 gm, and powder was mixed with 500 ml water at 60^0C for 20 minutes to form an aqueous extract of the plant (Vijayakumar 2018). The extract was filtered by using Whatman Filter paper No.1 and stored at 4^0C for further use (Datta 2017).

Green Synthesis of ZnO NPs: 60 ml of stored *L. lanata* plant extract was taken in a 250 ml conical flask which was kept at 70°C in a hot plate magnetic stirrer for 15 min. After this, 20mM Zinc acetate solution was added drop by drop while keeping it on magnetic stirrer at 70°C. After this the pH of solution was maintained by using an aqueous solution of NaOH. The formation of ZnONPs was observed from the turbidity in reaction solution. The reaction mixture centrifuge at 4000 rpm for 10 min. Dry the precipitate in the oven at 70 ^{0} C and stored in an airtight tube for further use.

Characterization of ZnO NPs: UV-VIS absorbance of ZnONPs in ultra-pure water were recorded by using UV-VIS spectrophotometer (JASCO.V-650) spectra analysis was carried out from 300-600 nm. The absorption peak at 300 - 400 nm gives primary information about the synthesis of ZnONPs (Gao 2020; Ahmed 2015). FT-IR spectra for ZnONPs were observed in the range of 4000 to 400cm^{-1} (NICOLET 6700, Thermo Fisher Scientific, Germany). The purity and percentage composition of ZnONPs was analyzed by EDX. The structural analysis and phase composition of the prepared product was identified X-beam diffraction (XRD, Bruker, D8 Advance, Germany) at room temperature. Field emission scanning electron microscopy (FE-SEM, Carl Zeiss, Ultra Plus) worked in the secondary electron mode, which was utilized to observe the surface microstructure and grain size of the prepared nanoparticles. The chemical composition of the representative samples was determined using energy-dispersive X-ray spectroscopy (EDX) attached joined with the FE-SEM system. HRTEM analysis was done to measure the diameter as well as to visualize the shape of synthesis AgNPs. The sample was dispersed in ethanol. A drop of thin dispersion was placed on "staining mat". Lacey Carbon coated copper grid was inserted into the drop with the coated side upwards. After about 30min sonicated, the grid removed and air dried for 30min then screened in JEOL 2100 TEM.

Antioxidant properties: The antioxidant activity of synthesized ZnONPs was observed by DPPH free radical scavenging assay (Jayappa 2020). DPPH is a stable free radical which is also a good scavenger for other radicals. Since the solution in methanol is of deep violet color. For which UV-VIS absorption is found at 517nm. When it accepts an electron from an antioxidant, its color becomes lighter and yellow (Mittal 2012). The equal volume of *L.Lanata* extract or NPs with various concentrations was mixed with 1ml of DPPH alcoholic solution and incubated it for 30min at room temperature. Ascorbic acid is used as standard antioxidant. For the absorbance of control, in place of extract, 1ml methanol was used(Rabiee 2020).

Results and Discussion

UV-VIS spectroscopy: UV-VIS absorption spectrum of synthesized ZnONPs is shown in figure (1). The UV-VIS absorption study Of ZnO nanoparticle observed at the wave length of 200 to 600 nm. In figure (4) three absorption peaks are observed at 270nm, 350nm and 397nm respectively. Out of these absorption peak at 350 and 397 nm are reporting strong evidence in the favor of presence of ZnONPs(Datta 2017). This absorption peak is due to surface plasma resonance.

XRD analysis

XRD pattern of synthesized ZnONPs clearly indicates crystalline structure due to presence of sharp and narrow diffraction peaks as shown in figure (2). The sharp diffraction peaks were observed at 2 θ values 36.39, 59.78, 73.30 and 77.48 degrees. These peaks are indexed as [111],[220],[331] and [222] diffraction lattice planes respectively. Which confirms the cubic structure for the ZnONPS matched with the JCPDS card number 01-077-0191. The Debye Scherrer's Formula can be used for calculating the crystalline size of the prepared nanoparticle. The Scherrer's Formula can be written as follows:

$$D = 0.9x\lambda/B \cos\theta$$

Where D is the crystalline size, λ is the wavelength of X-Ray used (0.15406 nm), beta is the full width at half maximum (FWHM), theta is the Bragg's angle. The average particle size of the prepared ZnO nanoparticle is 23.55 nm. Unassigned crystalline peaks at 42.4, 26.566, 20.788 degree are also recorded in the XRD

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pattern which is attributed to the presence of crystallized phytochemicals on the surface of L-ZnONPs which act as capping and stabilizing reagent (Ezealisiji 2019). The presence of phytochemicals also confirms by EDX results. EDX analysis : EDX spectra figure (3) for synthesized ZnONPs shows three peaks between 1 to 10 keV which were identify as Zinc 45.5% and oxygen 29.5%.

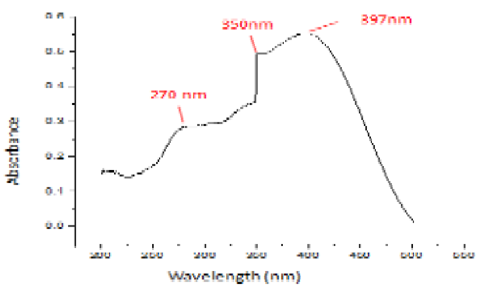


Fig 1. UV-VIS spectra for synthesized ZnONPs

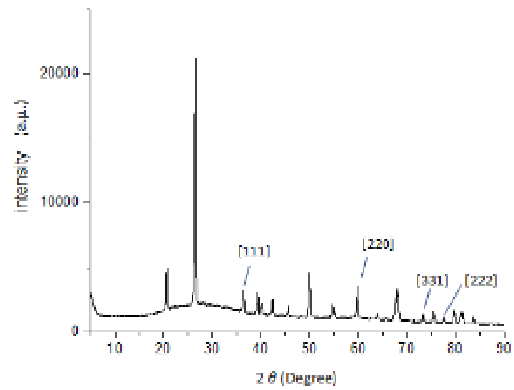


Fig 2. XRD pattern of synthesized ZnONPs

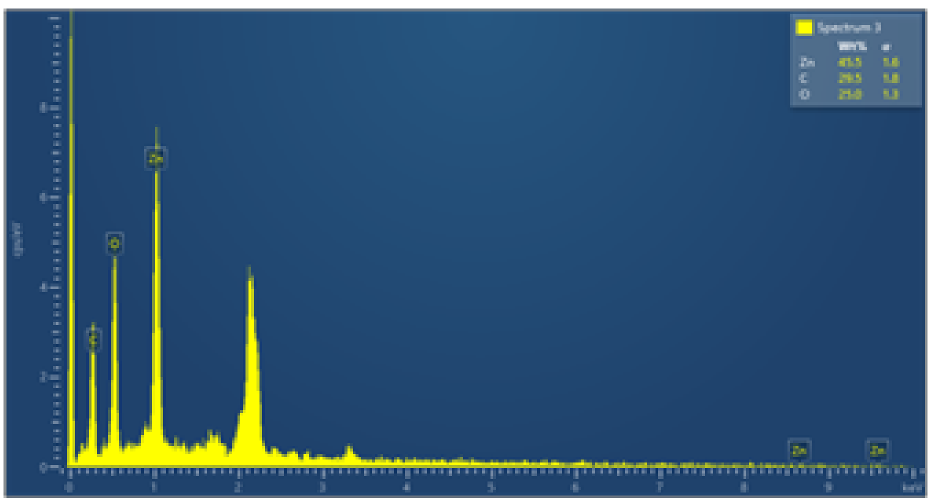


Figure 3. EDX spectra showing elemental composition of ZnONPs

The presence of C and P peak was originated from plant extract indicating the bioactive compound were absorbed on synthesis ZnONPs (Datta 2017; Bala 2015).

FESEM analysis “: FESEM image of synthesized ZnONPs shown in figure (4). The image of 20nm clearly showed high density spherical shaped nanoparticles with various particle size fig (4,a). They were also seen to be present in small Cluster form. Image J software was used to find out the diameter of the particle, in which 104 particles were taken. According to which the size of the ZnONPs particles is ranging from 12 to 37 nm and average particle size of ZnONPs is approximately 20 nm which is also approving by XRD and TEM results figure (5) (Datta 2017).

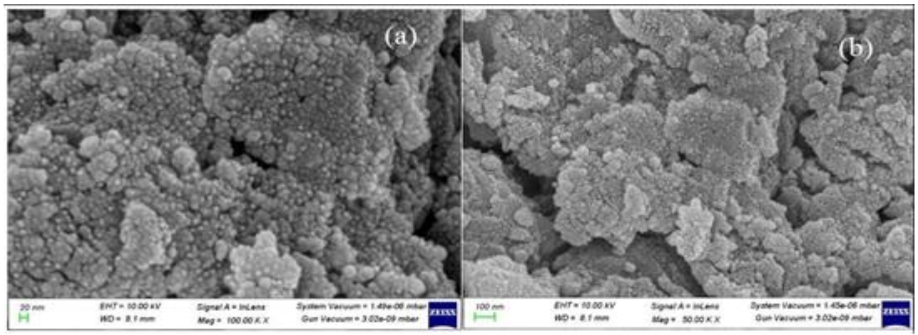


Figure 4. SEM images of synthesized ZnONPs

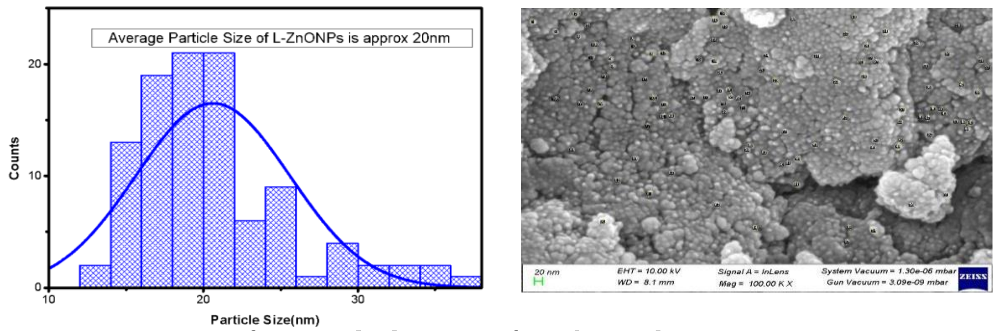


Figure 5. FESEM image for Particle diameter of synthesized ZnONPs

FT-IR analysis: The FT-IR spectrum of synthesized ZnONPs is shown in Fig 6. The peaks at 3411cm^{-1}, 2925cm^{-1},1618cm^{-1},1499cm^{-1},1410cm^{-1},1268cm^{-1},1048cm^{-1},796cm-1 and 610cm^{-1} were observed. Strong absorption peak at 3411 cm^{-1} was resulted from stretching of a -----OH group due to presence of phenol, alcohol and carbohydrate (Bala 2015; Alharthi 2021). The Peak at 2925 cm^{-1} and 1618 cm^{-1} are due to stretching vibration =C-H and C=C in aromatic compound. The peak at 1048 cm^{-1} contributed by skeletal C-O and C-C vibration bands of glycosidic and pyrenoid ring. However, 1499 cm^{-1} and 1410 cm^{-1} can be assigned to asymmetric and symmetric C=O stretching mode respectively. 610 cm^{-1} peak gives information about to the Zn-O (metal oxygen) vibration for L-ZNONPS (Singh 2019). These peaks showing the presence of phytochemicals on the surface of ZnONPs which confirm the presence of capping reagent on the surface of L-ZnONPs which supply by the *Leucas lanata* plant extract.

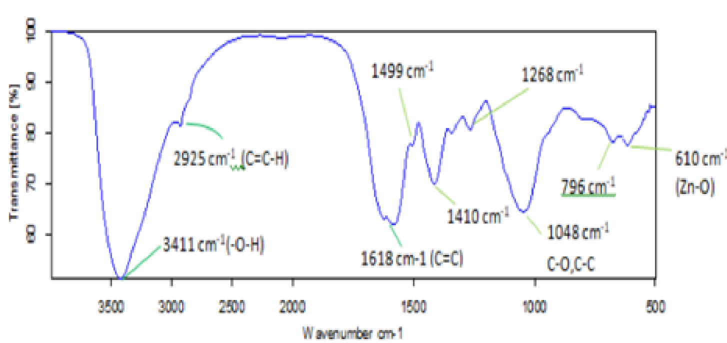
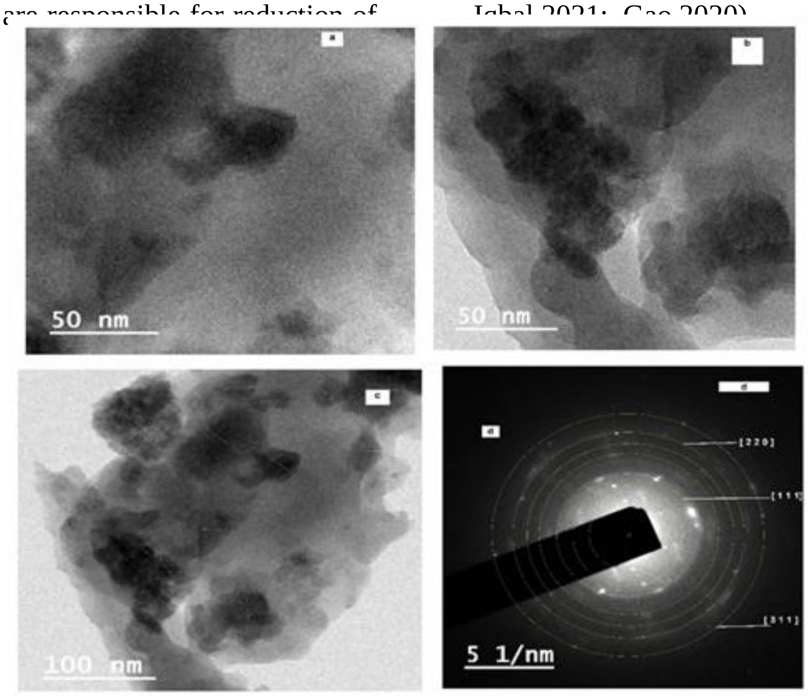


Fig 6. FT-IR spectra of synthesized ZnONPs

HRTEM Analysis: The TEM image of the ZnONPs is depicted in Fig 7. The size was in the range of 10 to 40 nm; the average size of the synthesized ZnONPs was found to be 19.65 nm (fig 7 c). The shape of ZnONPs was spherical and irregular with moderate variation in size shown in fig (7 a,b). The results of particle size and shape are supported by FESEM and XRD analysis. In order to verify the crystalline nature of the ZnONPs the selected area electron diffraction (SADE) patterns were obtained for the ZnONPs , for different regions and particles, as shown in fig(7,d) . The ring like diffraction pattern in SAED image indicate that particles are purely crystalline in nature and could be indexed on the basis of the cubic ZnONPs structure. The bright ring arises due to reflection from [111],[220]and [331] planes of cubic ZnO which supported by XRD results (Vidhu ,2014; Padalia 2015).

DPPH assay analysis

Plants contain specific metabolites that exhibit a range of purposeful activity.it is reported in literature that these metabolites or biomolecules of plants extract are responsible for reduction of metal ion and capping reagent in plant extract mediated synthesis of metallic nanoparticle. Antioxidant activity of ZnONPs was shown in terms scavenging capacity and % antioxidant activity. For this first of all 3.945 mg of DPPH free radical dissolve in 50ml of 99% methanol and it producing deep violet color. It is light sensitivity that is way covered the vessel by silver foil and place it at dark place. Make 1ml of working solution of plant extract, ZnONPs and Ascorbic acid(standard) of various concentration for example 100ug, 200ug, 300ug, 400gm and 500ug. Then add equal amount of DPPH solution in working sample solution. The incubated reaction mixture was incubated for half an hour at room temperature at dark place. Antioxidant activity was measured as color changes from violet to yellow. The % antioxidant activity vs concentration plot are shown in figure (7) it was clearly observed that the antioxidant activity increases on increasing concentration and ZnONPs was possesses high antioxidant activity as compared to *Leucas lanata*extract (Dai 2002; Siripireddy 2017; Iqbal 2021; Gao 2020).



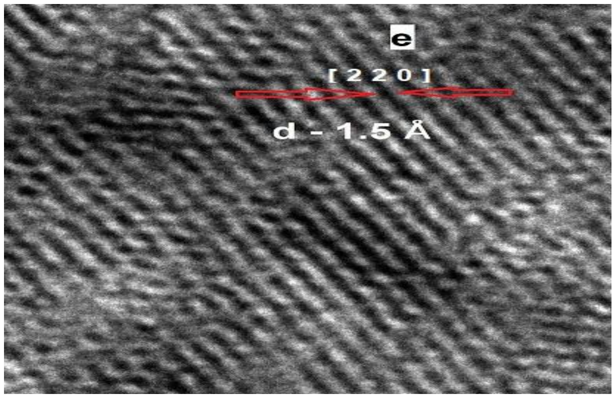


Figure 7. (a), (b) TEM images of synthesized ZnONPs in low and high magnification, (d) SAED patterns of the synthesized ZnONPs (e) d spacing value for [2 2 0] plane

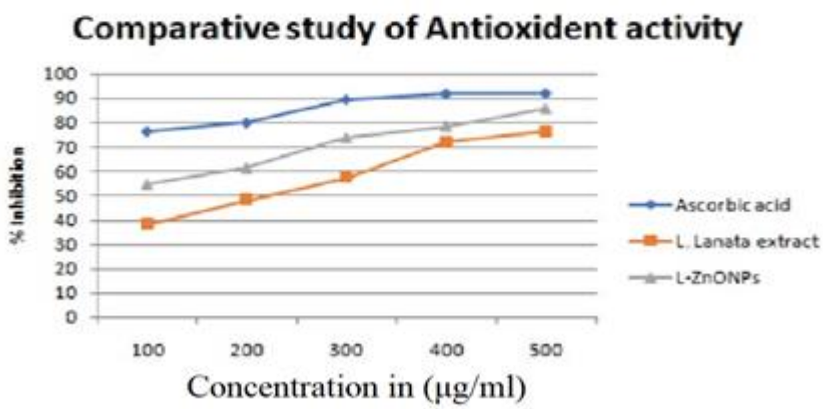


Figure 7. Comparative study of DPPH- Free radical scavenging Activity for Ascorbic acid, plant extract and ZnONPs

Conclusion

An aqueous plant extract of arial part of *Leucas lanata* plant was suitable for the synthesis of ZnO nanoparticle. This is the first report on the synthesis of ZnO nanoparticle from extract of *Leucas lanata* plant. XRD study showed cubic structure of ZnO nanoparticle. The crystalline size of synthesized ZnONPs was found in the range between 5 to 49 nm and average crystalline size is 23.5 nm showing by XRD analysis. The UV-VIS absorption confirmed that the maximum absorption at 350 to 397nm range corresponds to the intrinsic band gap of ZnONPs. FESEM analysis reported the spherical shaped of nanoparticles with average particle size 20 nm which is also supported by TEM results. FT-IR spectra revealed the absorption bands 3411cm^{-1} to 610 cm^{-1} confirming the important role of function groups involved in reduction of Zn^{+2} into ZnONPs. The purity of nanoparticles was confirmed by EDX data. Antioxidant activity of synthesized ZnONPs increased on increasing concentration. IC50 value for synthesized ZnONPs was 100 ug/ml which was higher than *Leucas lanata* plant extract. In brief, this is a simple, effective biosynthesis method which is full eco-friendly, cheaper, safe, non-toxic, simple as compared to physical and chemical methods.

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