ANTIMICROBIAL ACTIVITY OF BIOSYNTHESIZED ZINC OXIDE NANOPARTICLE FROM THE LEAF EXTRACT OF *LAURUS NOBILIS*

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Abstract

In recent years, the development of competent green chemistry methods for synthesis of metal nanoparticles have become a main limelight of researchers in the field of nanotechnology. Between the different biological sources, plants have been considered as the best candidate for the synthesis of metal NPs. The reasons behind choosing the plant as a best candidate among the biological sources is that plants can provide a superior platform for the synthesis of nanoparticles over chemical and biological methods, free from toxic chemicals, feasibility in cost competitive, faster rate of synthesis, protective, secondary metabolites can act as natural reducing agent as well as capping agent, and the nanoparticles from plants materials are more stable than other biological sources The current research work describes, zinc oxide nanoparticles (ZnONPs) synthesized by the leaf extracts of *Laurus nobilis.* The plant biomolecules induce the reduction of Zn+ ions to ZnONPs which also acts as a stabilizing agent. The prepared nanoparticles were characterized by using various analytical and spectroscopic tools such as UV visible spectroscopy, Fourier transform infrared spectroscopy (FT- IR), X-ray diffraction (XRD), Transmission electron microscopy (TEM) analysis and (EDAX). Along with this study we also investigate the antimicrobial activity of bio synthesized nanoparticles by using disc diffusion method against clinical and standard strains of the microbes *Vibrio Cholerae, Staph Aureus, and Candida Albicans*.

Keywords: Zinc Oxide Nanoparticles, Green Synthesis, Antibacterial Activity, Antifungal Activity, *Staph aureus, Vibrio Cholerae, Candida albicans*.

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Introduction

The synthesis of metal nanoparticles from plant extracts is considered an easy process relative to fungal/bacteria cultures since they require sterilized conditions and skills to preserve. Furthermore, the plant extracts used to synthesize the nanoparticles show various size and shape distributions. These nanoparticles have a large surface area to volume ratio, allowing for specific properties and behavior. One type of nanoparticle that has attracted attention is zinc and iron oxide nanoparticles due to their wide range of applications, including antibacterial, antifungal, antidiabetic, and antioxidant properties. The large excitation binding energy and wide bandgap (3.37 eV) of zinc oxide nanoparticles make them particularly promising for diverse applications in electronics and biomedical sciences (Jain *et al*., 2020). A wide variety of physical, chemical, and biological methods are available for the synthesis of nanoparticles. There are many processes for the synthesis of nano and micro-length scaled inorganic materials. Small nanoparticles can be reduced even during large-scale production. External experimental conditions like high energy, and high pressure are not required, causing significant energy saving (Herlekar *et al*., 2014). Biosynthesis of nanoparticles is an approach to synthesizing nanoparticles using microorganisms and plants having biomedical applications.

Green synthesis has advanced over chemical and physical methods as it is cost-operative, atmosphere-friendly, and easily scrabbled up for large-scale synthesis and in this method, there is no need to use high energy, temperature, and toxic chemicals. Green synthesis offers better influence, control over crystal growth, and steadiness. Green-synthesized nanoparticles are cheap and economical and have many applications in science (Naseem & Farrukh, 2015). NPs synthesized from a biomimetic approach show more catalytic activity and limit the use of expensive and toxic chemicals. For the synthesis of metal/metal oxide nanoparticles, plant biodiversity has been broadly considered due to the availability of effective phytochemicals in various plant extracts, especially in leaves such as ketones, aldehydes, flavones, amides, terpenoids, carboxylic acids, phenols, and ascorbic acids. These components are capable of reducing metal salts into metal nanoparticles (Prathna *et al*., 2010), (Doble *et al*.,2007). Green synthesis of metallic nanoparticles has been adopted to accommodate various biological materials (e.g., bacteria, fungi, algae, and plant extracts) (Valan Arasu, 2022). Among the available green methods of synthesis for metal/metal oxide nanoparticles, utilization of plant extracts is a rather simple and easy process to produce nanoparticles at a large scale relative to bacteria and/or fungi-mediated synthesis. These products are known collectively as biogenic nanoparticles (Annu *et al*., 2018).

Green synthesis of nanoparticles from leaf extracts

Plant extracts usually contain sugars, terpenoids, polyphenols, alkaloids, phenolic acids, and proteins, which are responsible for reducing and stabilizing metal nanoparticles. It has been confirmed that the functional groups, such as –C–O–C–, –C–O–, –C=C–, and –COOH, present in the phenolic compounds can assist in the formation of metallic nanoparticles (Sana *et al*., 2020).

Materials and Methods

Analytical grade Zinc Acetate Dihydrate and distilled water were purchased from S.A. Chemicals, Tirunelveli. In this study two leaves were used in the nano synthesis of zinc oxide nanoparticles. The bay leaves (*Laurus nobilis*) (ZnB) were freshly collected from the home garden of the author at Monday market, Kanyakumari district.

Preparation of Leaf extracts

Collected fresh bay leaf was thoroughly washed using tap and distilled water to remove the dust particles and sun dried to remove the residual moisture. The dried leaves were ground into a coarse powder using mortar and pestle. The aqueous extracts of the sample were prepared by boiling 20 g of the powdered leaves in 200 ml of double distilled water at 80°C for 60 minutes while stirring using a magnetic stirrer at 800 rpm. The extract was then cooled to room temperature and filtered using Whatman No.1 filter paper and stored in a refrigerator at 4°C for further experimental use.

Biosynthesis of ZnO Nps

Zinc Acetate Dihydrate was used as the precursor for the synthesis of the ZnO Nps. 20ml of the extracts were added drop wise with 50ml of 0.5N $Zn(CH_3COO)_2.2H_2O$ solution. Simultaneously, the mixture is heated in a rotamantle for about an hour and thirty minutes. The resultant mixture was stirred using a magnetic stirrer. This shows the formation of a white colored precipitate, which confirms the synthesis of Zinc oxide nanoparticles. After letting the solution to cool, it is then filtered through suction using Whatman No.1 filter paper and subsequently washed with Ethanol and water

for 2 to 3 times. The resultant product is then dried in a hot air over at 120 ºC for 3 hours and stored in a seal tight container for further use.

Analytical Methods

FT-IR spectra of the sample was recorded on a FT-IR spectrometer (SIC, Sivakasi). XRD patterns were recorded by an X-ray Diffractometer at Alagappa University, Karaikudi from a broad focus Cu tube operated at 40kv and 40MA. The morphology of nanoparticles was analyzed at HRTEM facility, PSG Institute of Advanced Studies, Coimbatore. The UV-VIS spectra of the sample to measure the absorbance were done by using the double beam UV- 2400 PC series spectrometer (SIC, Sivakasi). Antibacterial and antifungal activity were examined by the Kirby-Bauer method based on the zone of inhibition and minimal inhibitory indices (MIC) at Scudder Lab, Nagercoil.

Results and Discussion

Optical Analysis of ZnO Nps

Adding zinc acetate dihydrate to the extracts leads to physio-chemical changes in the aqueous solution. The most prominent of which is change in the color of the reaction mixture that can be observed within few minutes. The appearance of white precipitate was considered as an initial signature to formation of NPs. Flavonoids and phenolic compounds are thought to be responsible for Zn ions to ZnO Nps. A clear illustration of change in color of the reaction mixtures into white precipitate is due to formation of ZnO NPs. Temperature is considered an important contributing factor in synthesis of good-sized nanoparticles. It is also well established that higher the temperature of reaction process of NPs synthesis, the smaller the size of the NP. Therefore, we use a relatively higher temperature of 80 °C for incubating the reactants that leads to the production of very small sized ZnO NPs.

Surface Morphology

The synthesis of ZnO NPs was further examined by UV spectrophotometry by measuring the absorbance in the range of 220-900 nm. Figure (1) shows the UV peaks recorded by the spectrophotometer. The maximum absorption peak for ZnO NPs synthesized via the leaf extracts of bay (ZnB) was recorded at 358.88 nm, that further verified the formation of ZnO NPs. Firstly, the results satisfy standard ZnO absorption pattern because all oxide materials have wide band gaps and tend to have shorter wavelengths. Moreover, if the material is of nanoscale, it tends to have further shorter wavelength.

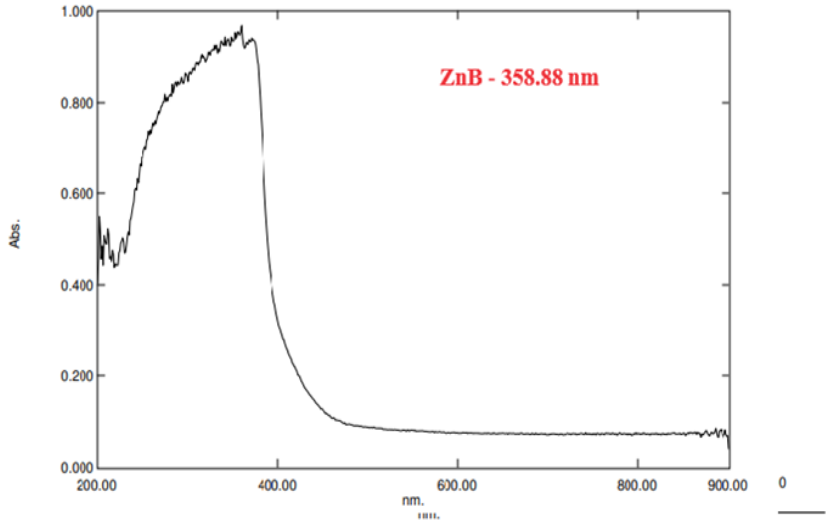

Fig 1: UV spectrum of ZnB

X-Ray Diffraction Analysis (XRD)

The X-ray diffraction pattern of zinc oxide nanoparticles shows definite line broadening of the X-ray diffraction peaks, showing that the prepared particles were in the nanoscale range, (Faisal S *et al.*, 2021) as shown in figure (2). ZnO NPs showed diffraction peaks with 2θ values identified at 36.2548º which corresponds to the plane 101 and with the crystallite size 47.55nm. The diameter of zinc oxide crystallites was calculated by the Debye−Scherrer formula, [$D = K\lambda / \beta Cos\theta$] where D is mean crystallite size of the powder, θ is the

Bragg diffraction angle, β is the full width at half-maximum, λ is the wavelength of Cukα and k is a constant (Rossi L *et al*.,). These results indicate that the amount of extract used strongly influenced the crystallinity and the size of the crystals.

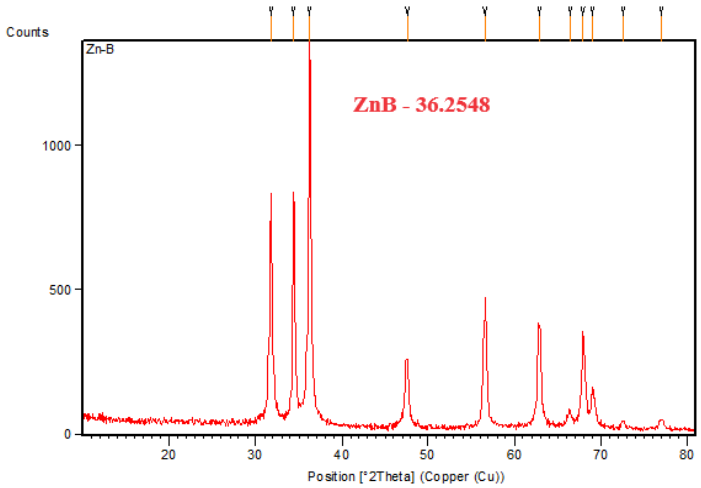

Fig 2: XRD spectrum of ZnB

FT-IR Spectroscopy

One of the key instrumental techniques for characterizing nanoparticles is Fourier transform infrared spectroscopy. An FT-IR spectrum exhibits the possible adherent organic complex helping in stabilization and capping of Zinc oxide nano-particles. This method depends on absorbing light molecules, measures the vibrations of atoms and determines functional groups and structure of compounds in a molecule (Khan Z *et.al*, 2019).

In addition to functional group determination, FTIR spectroscopy can also be used to evaluate the infrared emission spectrum, absorption, photoconductivity, or Raman scattering of the nanoparticles. These spectroscopic techniques provide a fingerprint of the nanoparticles, consisting of absorption peaks that correspond to the frequencies of vibrations between the bonds of atoms in the nanoparticle. This information is critical for identifying the types of functional groups present in the nanoparticles and can aid in the synthesis and characterization of nanoparticles using green technology. Furthermore, Fourier transform infrared spectroscopy is a powerful tool for characterizing the surface chemistry of nanoparticles (Christopher *et al*., 2015). By using FTIR spectroscopy, researchers can detect organic functional groups attached to the surface of nanoparticles and other surface chemical residues. This information allows for a deeper understanding of the surface chemistry of nanoparticles and can provide insights into their stability, aggregation behavior, and potential interactions with surrounding molecules

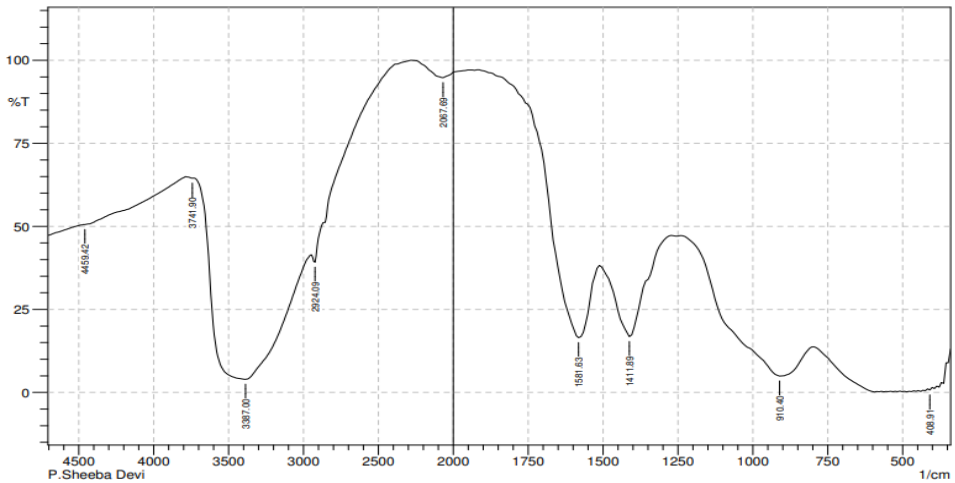

Fig 3: FT-IR spectrum of ZnB

Functional Group	Frequency ranges of Synthesised ZnO Nanoparticles (ZnB)
Phenols (Ar-OH)	3387.00
Diketones (C=O)	1581.63
Alkanes (C-H)	1411.89
Aromatic compound (C=C)	910.40
Halogens (C-Cl)	550.25
Alkyl Halides (R-X)	408.91

Table 1: FT-IR spectroscopy peak values and functional groups corresponding to the synthesized ZnO nanoparticles.

To identify the functional groups associated with the ZnONPs formation, FTIR spectrometry was performed and the corresponding frequency ranges were shown in table 1. A broad absorption peak in the range of 3387cm^{-1} is present in the fig 3, which can be attributed to the characteristic absorption of phenolic (O–H) groups. The peak at 408 cm^{-1} is due to the presence of Zn–O bond (Srivastava *et al*., 2013). Some unresolved peaks in precipitate may be due to some impurities.

Transmission Electron Microscopy

The uniform distribution and almost spherically shaped ZnO NPs can be observed in the TEM image as shown in Figure 4. TEM images of the nanoparticles confirm the formation of ZnO nanoparticles in the range of 50-100nm. The NPs were dispersed in ethanol and sonicated in an ultrasonic bath for 20 min for TEM analysis. The TEM image in Figure 4 clearly shows that the NPs are mostly hexagonal with a size ranging from 25 to 30 nm. The selected area electron diffraction pattern, depicted in figure 4, confirms the polycrystalline nature of the biosynthesized ZnO NPs. Smaller and spherical size nanoparticles with larger surface area show significant activity in various fields. The smaller size and well dispersed ZnO-NPs show significant activity, especially in the field of medical, chemo and electrochemistry (Khan Z *et.al*, 2019).

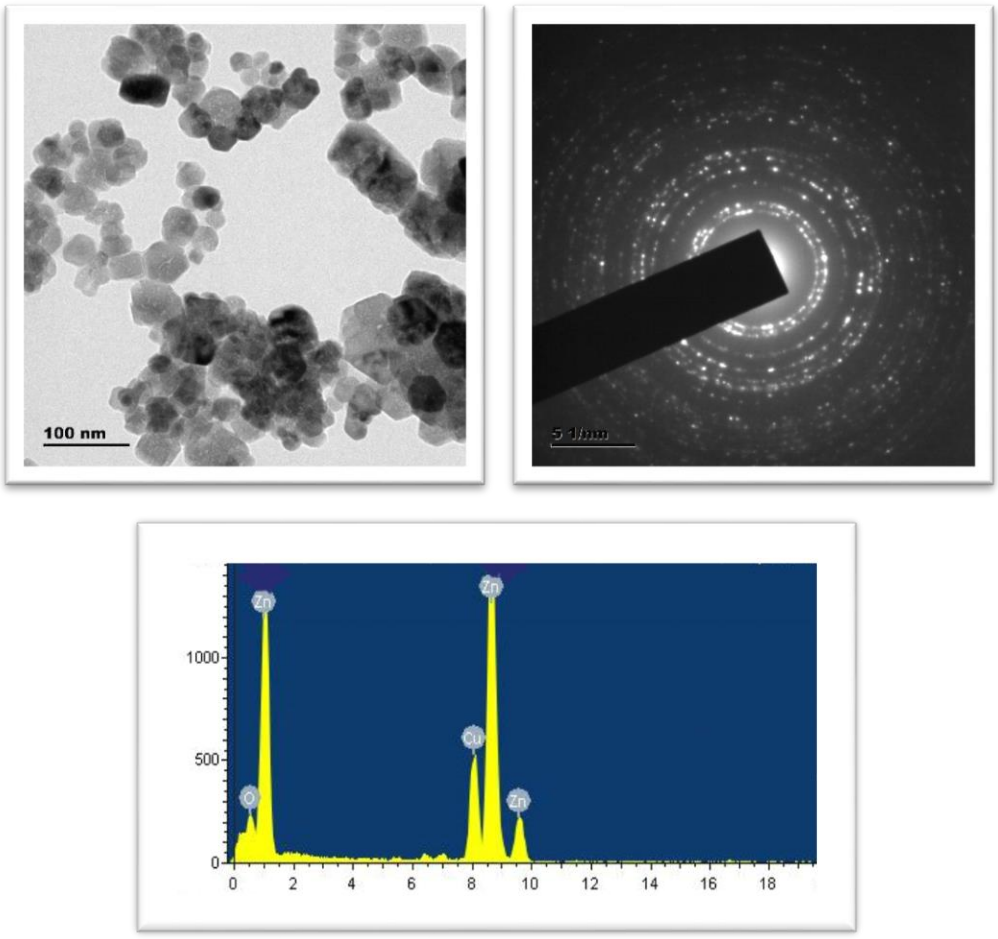

Figure 4: Pictorial representation of TEM, EDAX and Electron Diffraction patterns of biosynthesized ZnO nanoparticles from ZnB.

The EDAX of the NPs reveals that there is clearly the formation of zinc oxide nanoparticles. The elemental composition of ZnO-NPs (table 2) was confirmed with EDX which exhibited strong signals for ZnO-NPs as given in Figure 5. EDAX results reveal that all the ionic Zn was resulted into the formation of ZnO-NPs. The EDX pattern clearly shows that the reduction of Zn solution with the extracts yielded ZnO-NPs which are crystalline in nature.

Elements	**Zn (K)**		**O (K)**	
	Weight %	**Atomic %**	**Weight %**	**Atomic %**
ZnB	76.24	73.23	1.12	4.39

Table 2: Elemental composition of ZnB nanoparticles

Antibacterial and Antifungal Activities of ZnONPs

The prepared Zinc oxide nano-particles have excellent anti-bacterial activities against Gram-Negative bacteria, *Vibrio Cholerae* and Gram-Positive bacteria, *Staph aureus*. The inhibition zone (fig 5) against *Vibrio Cholerae* and *Staph aureus* were exhibited to a greater extent of about +1mm and +4mm on comparison with standard Amikacin (21mm and 17mm) respectively. Thus, the results (table 3) clearly shows that biosynthesized ZnO-NPs have substantial anti-bacterial activity. The antibacterial activity may involve the accumulation of ZnO NPs in the outer membrane or cytoplasm of bacterial cells and trigger Zn^{+} release, which would cause bacterial cell membrane disintegration, membrane protein damage, and genomic instability, resulting in the death of bacterial cells (Dutta *et al.*, 2013). The antibacterial mechanism of ZnO NPs involves the direct interaction between ZnO nanoparticles and cell surfaces affecting cell membrane permeability; afterwards these nanoparticles enter and induce oxidative stress in bacterial cells, which results in the inhibition of cell growth and eventually cell death; the demonstrated antibacterial activity of ZnO NP recommends its possible application in the food preservation field. It can be applied as a potent sanitizing agent for disinfecting and sterilizing food industry equipment and containers against the attack and contamination with food borne pathogenic bacteria (Sabir *et al.*, 2014).

Zinc oxide nanoparticles also displayed antifungal activities against *Candida Albicans*. The antifungal effect of NPs is dependent on their size and concentration; possibly, an increase in NPs' concentration may increase the inhibition of fungal growth, due to burst of fungal cell membrane, and cause a decline in the fungal enzymatic activity (Kim *et al*, 2012). From the current study, ZnO NPs obtained using Bay leaf extract showed significant antimicrobial activity of +6mm against the fungal pathogens *Candida Albicans* compared to the standard Nystatin (13mm).

Fig 5: Zone of Inhibition of the synthesised ZnO Nanoparticles against *Vibrio Cholerae*, *Staph Auerus* and *Candida Albicans* respectively.

Microbes	**ZnB**	**Control (*Amikacin*)**
Vibrio Cholerae	22mm	21mm
Staph Aureus	21mm	17mm
Candida Albicans	19mm	**Control (*Nystatin*)** 13mm

Table 3: Antibacterial and Antifungal activity of ZnO nanoparticles

Conclusion

In the present study the fabrication ZnO nanoparticles was carried out by green synthesis. The *Laurus nobilis* (Bay) leaves extract was used effectively for the above synthesis. The biological synthesis of zinc nanoparticles using leaf extract of *Laurus nobilis* provide an environmentally friendly, simple and efficient route for synthesis of nanoparticles. The use of plant extract avoids usage of harmful and toxic reducing and stabilizing agents. The characterization of ZnO nanoparticles were carried out using different techniques like XRD, FTIR, TEM, EDAX, and UV–Vis etc. The ZnO NPs was highly active against *V.Cholerae*, *S.aureus* and *C.albicans*

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