Synthesis and characterization of *Morinda citrifolia* leaf extract capped Zinc Oxide nanoparticles and development of antibacterial activity using co-precipitation method.

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Abstract

Nanotechnology is relatively a new branch of science that has found a wide range of applications which ranges from energy production to biomedical applications. In present study, ZnO nanoparticles are synthesized using fresh leaf extract *Morinda citrifolia* by Co-precipitation method. The synthesized nanoparticles are characterized by X-Ray diffraction analysis (XRD), Fourier Transform Infrared spectral analysis (FTIR), UV-Visible spectral analysis (UV-Vis), Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray analysis (EDX) and Antimicrobial activity. From XRD, the particle size of ZnO Nps is determined and is about 15.77 nm. The presence of biomolecules and functional groups are investigated by FTIR analysis. The band gap energy of ZnO Nps is estimated using UV-Vis Spectral analysis and it is approximately 3.31eV. The surface morphology and elemental composition of ZnO Nps is observed under SEM and EDX. The inhibition zone of ZnO Nps of various bacteria and fungi is determined by antimicrobial activity and results increasing zone of inhibition for various concentration of sample.

Keywords: Nanotechnology; Green synthesis; Zinc oxide nanoparticle; *Morinda citrifolia*; Antibacterial activity

1. Introduction

Nanotechnology deals with the study of controlling the matter on an atom and molecule. Generally a nanotechnology deals with particle size between 1-100 nanometres and makes the material lighter, stronger, faster, smaller and more durable. Modern science was based on the unifying features at nanoscale range which contributes a new foundation for innovation, knowledge and integration of technology [1]. Recently the nanotechnology was an active field of biological sciences research [2] . The nanotechnology has various applications in the fields of science as diverse as surface science, organic chemistry, molecular biology, semiconductor physics, micro fabrication, etc.[3]. Traditionally, nanoparticles have been synthesized via different physical and chemical methods, such as microwave irradiation, ultrasonication, laser vaporization, solid-state thermal decomposition, hydrothermal, and Sol-gel [4–7]. These methods require high energy, expensive equipment, and space. Additionally, the absorption of some toxic chemicals on their surface makes toxic for the environment and also unsuitable for biological applications [8–10]. The advantage of green synthesis of NPs was safe, low cost, and eco-friendly when compared to the conventional methods [11], [12]. Additionally, the use of nontoxic extracts as reducing and stabilizing agents was also an additive advantage [13]. Because of its contents (many bioactive molecules such as polyphenols, enzymes, polysaccharides, esters, and terpenoids), plant leaf extracts may act as reducing and stabilizing agents in the nanoparticles synthesis processes [14–17]. Zinc oxide was an inorganic compound and was used as an additive in numerous materials and products including cosmetics, food supplements, rubbers, plastics, ceramics, glass, cement, lubricants, paints, ointments, adhesives, sealants, pigments, foods, batteries, ferrites, fire retardants, and first-aid tapes. ZnO nanoparticles have tremendous potential in biological applications such as biological assays, biological labeling, antibacterial and antifungal activities, drug delivery, and especially as nanoparticles. *Morinda* was extensively used in traditional medicine for the treatment of leprosy, jaundice, asthma, bronchitis, skin eruptions, burns, tongue sores, earache, indigestion, eye infections, nausea, insect bites, and fever. Phytochemical studies reveal the presence of phenols, tannins, saponins, terpenoids, flavonoids, arabinose, xylose, mannose, galactose and glucose. Studies on the plant extract particularly the leaf extract shows that it possesses antihyperglycemic, xanthine oxidase inhibitory, analgesic, anti-inflammatory, and antipyretic, antioxidant, antihyperlipidemic, antimicrobial, anti-hepatotoxic and anti-insecticidal activities. The plant leaf extract also shows significant activity chemoprotective effect against cyclophosphamide, commonly used in the treatment of cancer and autoimmune diseases [18]. In the present investigation report the synthesis and characterization of Zinc Oxide nanoparticles using *Morinda citrifolia* leaf extract and was characterized by XRD, FTIR, UV-Visible, SEM, EDX and antibacterial activity.

2. Materials and methods

2.1 Preparation of leaf extract

Fresh leaves of *Morinda citrifolia* (noni) were collected from Dharmapuri district, Tamil Nadu, India. The 30 grams of leaves were taken and thoroughly washed with double distilled water. The 10ml of leaf extract was mixed with 50 ml of distilled water and it was stirred for 30 minutes. Then, the leaf extract was filtered using whatman no 1 filter paper and was used for nanoparticles synthesis.

2.2 Preparation of zinc oxide nanoparticles

0.4M of zinc acetate dihydrate solution is prepared by 50 ml of double distilled water and stirred for 30 minutes. The collected leaves extract was added to the zinc acetate dihydrate solution and were allowed to stir for one hour. The pH of the solution was maintained at 12 by adding sodium hydroxide pellets. The color change from green to pale white was observed. The solution gets precipitated and washed three times using double distilled water inorder to remove impurities. Then the solution was dried using hot air oven at 100˚C for 2 hours and using muffle furnace at 400˚C for 3 hours. The particle was grinded using mortar to get fine zinc oxide nanoparticles.

3. Characterization techniques

The characterization of materials was significant when it comes to understand their overall properties. In order to introduce nanomaterials to various applications, detailed characterization of their optical, morphological, electrical, thermal and magnetic properties were needed. *Morinda citrifolia* leaf extract capped ZnO nanoparticles have been synthesized using Co-Precipitation method. The particle size, presence of biomolecules, band gap energy, surface morphology, and elemental composition are characterized by X-Ray diffraction analysis (XRD), Fourier Transform Infrared spectral analysis (FTIR), UV-Visible spectral analysis (UV-Vis), Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray analysis (EDX). Agar well diffusion method is followed to determine an antibacterial activity. It is investigated against both Gram positive and Gram negative bacteria and the zone of inhibition is measured.

4. Result and Discussion

4.1 X-Ray Diffraction analysis

The crystalline size of the synthesized ZnO nanoparticles was determined by X-ray diffraction analysis method. **Figure 1** shows the X-ray diffraction peaks of ZnO nanoparticles which was prepared by co-precipitation method [19]. Typical XRD pattern of zinc oxide was found by Bragg reflections corresponding to (100), (002), (101), (102), (110), (103), (200) sets of lattice planes. The peaks were corresponds to hexagonal wurtzite phase of ZnO (JCPDS card no: 79-2205) and also further confirms the synthesized nanoparticle has free of impurities as it does not contain any characteristic XRD peaks other than ZnO peaks. The present study clearly indicates that the X-Ray diffraction pattern of biologically synthesized zinc oxide nanoparticle was crystalline in nature.

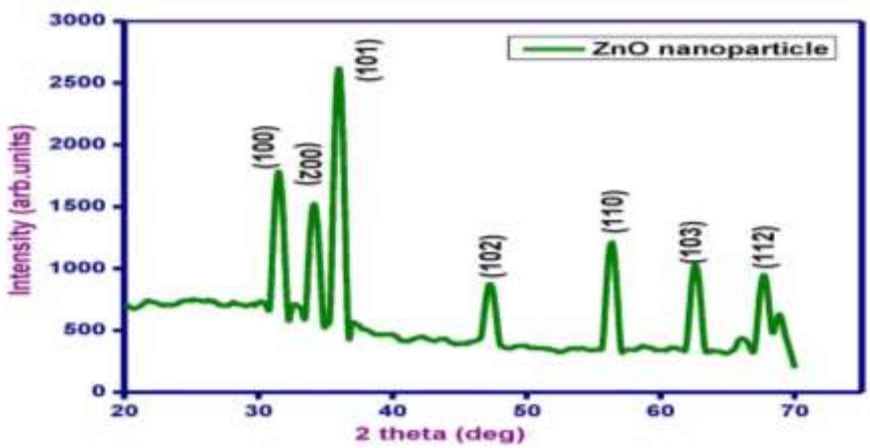

Figure 1: XRD Pattern of Green synthesized ZnO nanoparticles using *Morinda citrifolia* leaf extract

From XRD patterns analysis, the peak intensity, position width, and full-width at half-maximum (FWHM) data were determined. The average particle size was calculated by Debye Scherrer's formula [20].

$$D = \frac{k\lambda}{\beta\cos\theta}\ \text{nm}$$

Where, k = Scherrer's constant, λ = wavelength of X-rays used, θ = Bragg diffraction angle, β = full width at half-maximum (FWHM) of the diffraction peak. A definite line broadening of the diffraction peaks was an indication that the synthesized ZnO nanoparticles were in nanometer range [21]. The particle size of synthesized ZnO nanoparticles using *Morinda citrifolia* leaf extract was 15.77 nm. The crystalline size and d_{hkl} values are depicted in **Table 1.**

Table 1: Crystalline size and hkl values of observed crystalline peaks

S.No	2θ (Degree)	d (A°)	FWHM (deg)	Intensity	d_{hkl}	Crystalline size (nm)	Average crystal size (nm)
1.	31.7819	2.81329	0.53100	59	100	15.55	15.77
2.	34.4231	2.60324	0.49270	47	002	16.87	
3.	36.2569	2.47567	0.54390	100	101	15.36	
4.	47.5577	1.91043	0.5811	20	102	14.93	
5.	56.6066	1.6246	0.5534	35	110	16.30	
6.	62.8454	1.4768	0.5856	28	103	15.89	
7.	67.9659	1.3781	0.6197	23	200	15.46	

4.2 FTIR spectral analysis

FTIR spectral analysis was performed to identify the possible biomolecules for capping and for proficient stabilization of zinc oxide nanoparticles which was synthesized by *Morinda citrifolia* leaf extract. The FTIR spectrum of ZnO nanoparticles were measured in the range from 4000 to 400 cm^{-1}. **Figure 2** shows FTIR spectrum of synthesized ZnO nanoparticles using *Morinda citrifolia* leaf extract. The FTIR spectrum of ZnO nanoparticles shows strong peaks at 3388.97cm^{-1}, 2928.46 cm^{-1}, 2342.31 cm^{-1}, 1574.83 cm^{-1}, 1389.30 cm^{-1} , 893.11 cm^{-1} and 508.33 cm^{-1}. The broad band at 3388.97 cm^{-1} represents O-H stretching vibration. A prominent level of absorption peak was observed at 2928.46 cm^{-1} reveals the presence of C-H stretching vibrations of an aromatic aldehyde. The absorption peak at 1574.83 cm^{-1} and 1389.30 cm^{-1} shows stretching vibration of C-C alkanes. The peak at 893.11 cm^{-1} was attributed to the presence of C-H bending vibration [22].The peak at 508.33 cm^{-1} confirms the presence of zinc oxide nanoparticles. The leaf extract contains a lot of biomolecules such as tannins, alcohols, aldehydes, ketones,as well as phytochemical constituents namely flavonoids, amino acids, phenolic compounds, steroids possessing antibacterial and anticancer activity [23]. These vibrations confirms the presence of bioactive compounds in the synthesized ZnO nanoparticles using fresh leaf extract *Morinda citrifolia* [24].

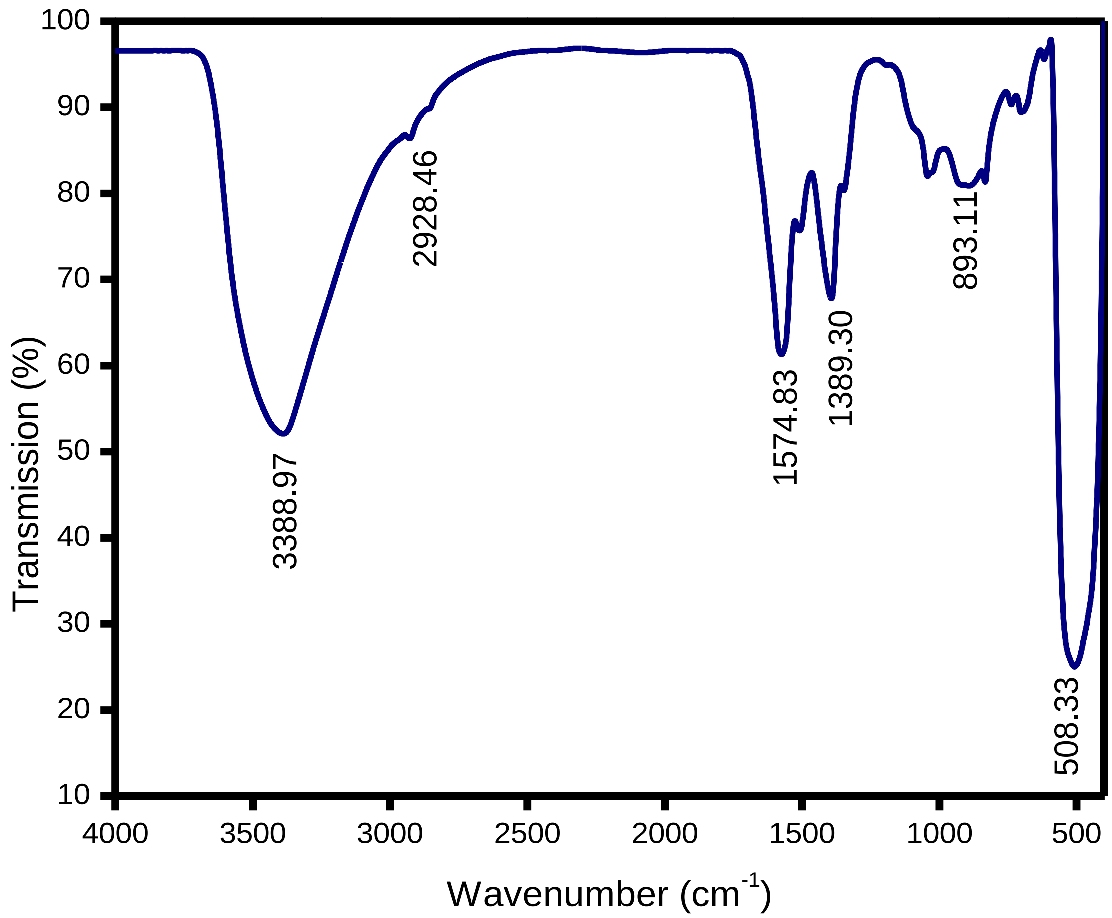

Figure 2: FTIR spectrum of ZnO nanoparticles using *Morinda citrifolia* leaf extract

4.3 UV-Visible spectral analysis

The formation and stability of zinc oxide nanoparticles in aqueous solution are confirmed by using UV-Visible spectral analysis in the range from 300 to 800 nm. The band gap energy due to the energy transfer spectra was calculated using formula,

$$E = \frac{hc}{\lambda} eV$$

The plant extract was mixed with aqueous solution of zinc acetate dihydrate and stirred in a magnetic stirrer. The solution started to change color from light green to pale white color. This confined the formation of zinc oxide nanoparticles was pure in nature**.**

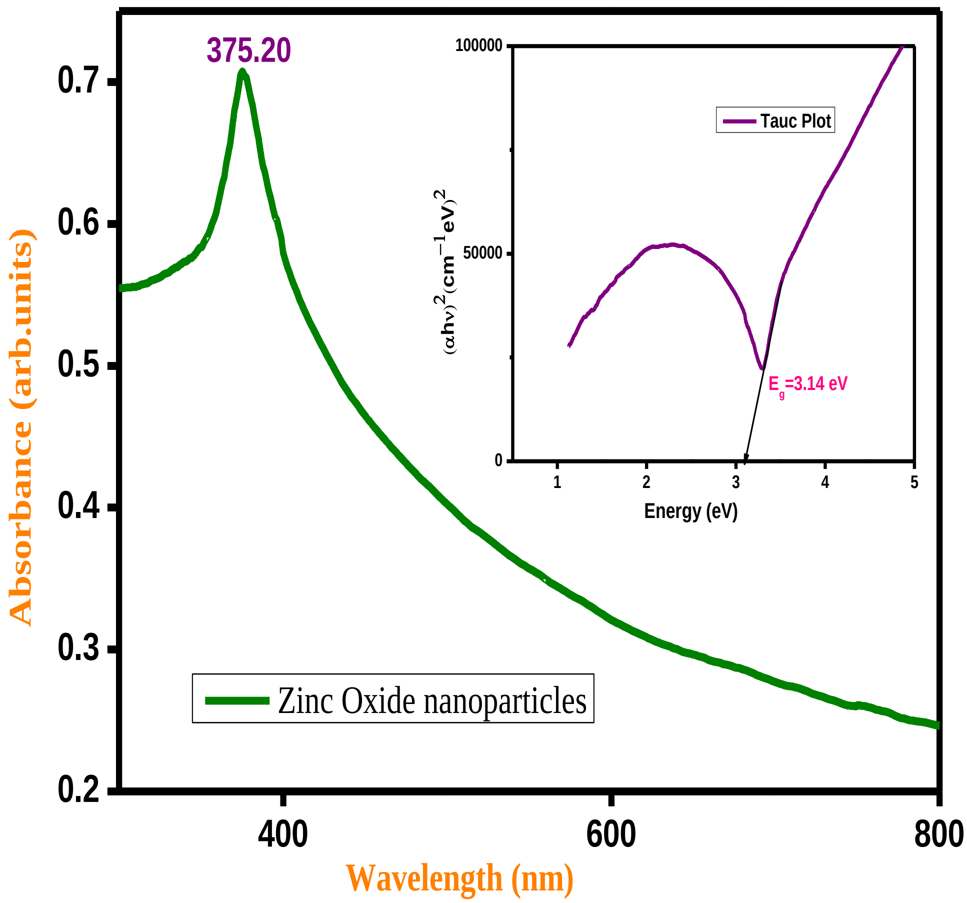

Figure 3: UV-Visible absorption spectrum of Zinc Oxide nanoparticle using *Morinda citrifolia* leaf extract

Figure 3 shows the UV-Visible spectrum of ZnO nanoparticles using *Morinda citrifolia* leaf extract. The UV-Visible Spectrum was recorded for zinc oxide nanoparticles using *Morinda citrifolia* leaf extract. The result shows optical absorbance peak within the range around 375.20 nm and the band gap energy was 3.31 eV which are given in **Table 2**.

Table 2: UV – Absorbance Spectrum of Zinc Oxide nanoparticles using *Morinda citrifolia* leaf extract

Sample	h (Js)	C (m/sec)	λ (nm)	Band gap (eV)	
				Experimental	Theoretical
Biosynthesized Zinc Oxide nanoparticles using *Morinda citrifolia* leaf extract	6.626×10^{-34}	3×10^{8}	375.20	3.31	3.14

4.4 Morphological studies

The surface morphology, size and shape of zinc oxide nanoparticles were analyzed by Scanning Electron Microscope. The **Figure 4** shows the morphology of synthesized zinc oxide nanoparticles of *Morinda citrifolia* leaf extract. Predominantly the synthesized ZnO nanoparticles were hexagonal in shape. A closer look shows the presence of several nanoparticles were aggregated and some individual crystals were clearly visible. The surface size of the synthesized ZnO nanoparticles is 117 nm.

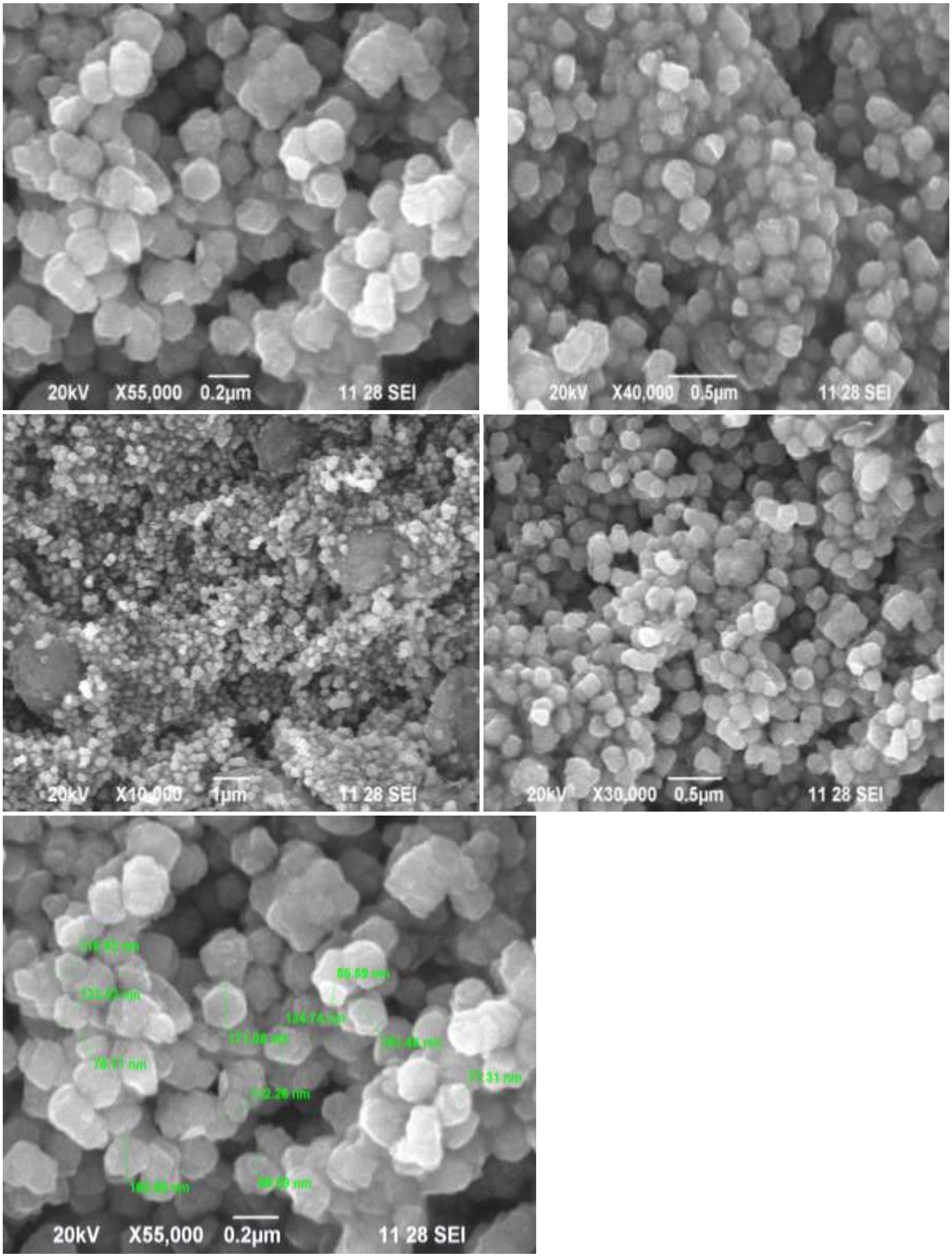

Figure 4: SEM images of zinc oxide nanoparticles using *Morinda citrifolia* leaf extract

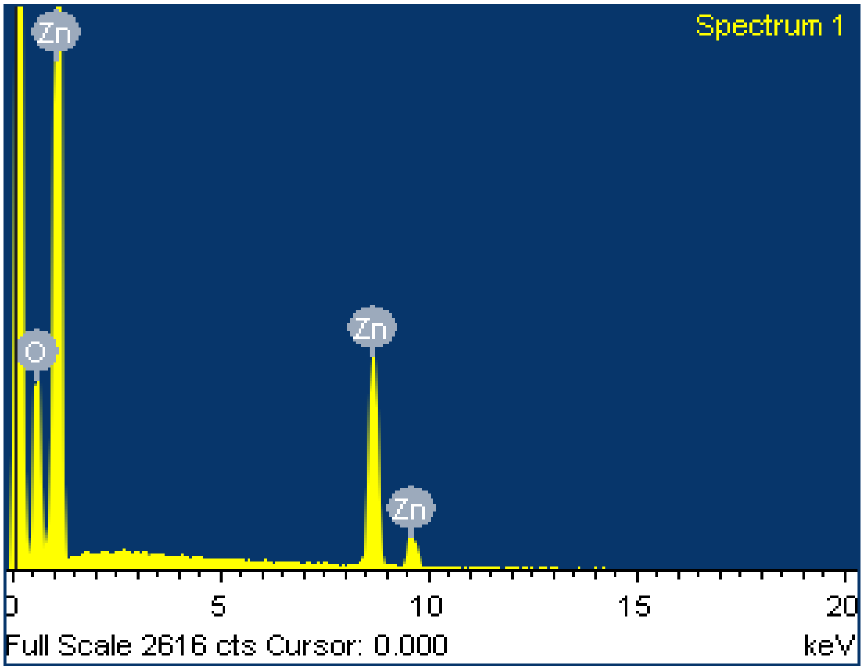

Figure 5: Compositional analysis of Zinc Oxide nanoparticles using *Morinda citrifolia* leaf extract

Energy dispersive X-Ray analysis (EDX) was mainly used to determine the elemental composition of a sample. The stoichiometric mass percentage of zinc and oxygen are 72.55 and 27.45 respectively and is shown in **Table 3**. **Figure 5** shows the compositional analysis of zinc oxide nanoparticles using *Morinda citrifolia* leaf extract.

Table 3: EDX analysis for Zinc Oxide nanoparticles using *Morinda citrifolia* leaf extract

S.No	Sample	Wt. %of Zinc	Wt. % of Oxide
1.	zinc oxide nano particles	72.55	27.45

4.5 Photoluminescence Spectrum

Photoluminescence spectroscopy is a contactless, nondestructive method of probing the electronic structure of materials and it is widely used technique for characterization of the optical and electronic properties of semiconductors and molecules [14, 15]. Photoluminescence is the spontaneous emission of light from a material under optical excitation. The excitation energy and intensity are used to probe different regions and excitation concentrations in the sample. The strongest spectrum is observed in 644.45 nm indicates red emission of light is shown in **Figure 6**.

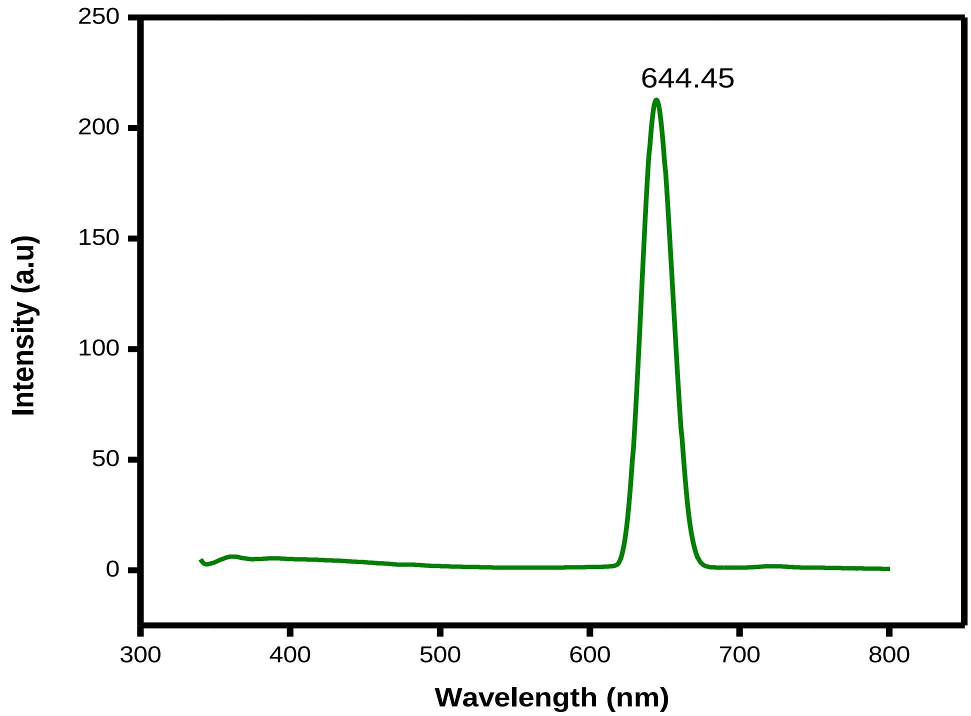

Figure 6: Photoluminescence Spectrum of ZnO nanoparticles

4.6Antibacteriall activity

The antibacterial activity of the biosynthesized zinc oxide nanoparticles using leaf extract of *Morinda citrifolia* has potential against both gram positive and gram negative bacteria on human pathogens with various concentrations as suggested by the diameter of inhibition zone. **Figure 7** shows the antibacterial activity of biosynthesized ZnO nanoparticles from leaf extract of *Morinda citrifolia*. The maximum zone of inhibition was observed for 10 mm for 100 µg/ml concentrations for *S. aureus*. The minimum zone of inhibition was observed for 5mm for 100µg/ml concentrations for *Ps. aeruginosa*. By comparing the gram negative bacteria, the gram positive bacteria has highest zone of inhibition.

Figure 8 shows the zone of inhibition against gram positive and gram negative bacteria. The zone of inhibition obtained by ZnO nanoparticles are shown in **Table 4**.

Figure 8: Antibacterial activity of ZnO nanoparticles

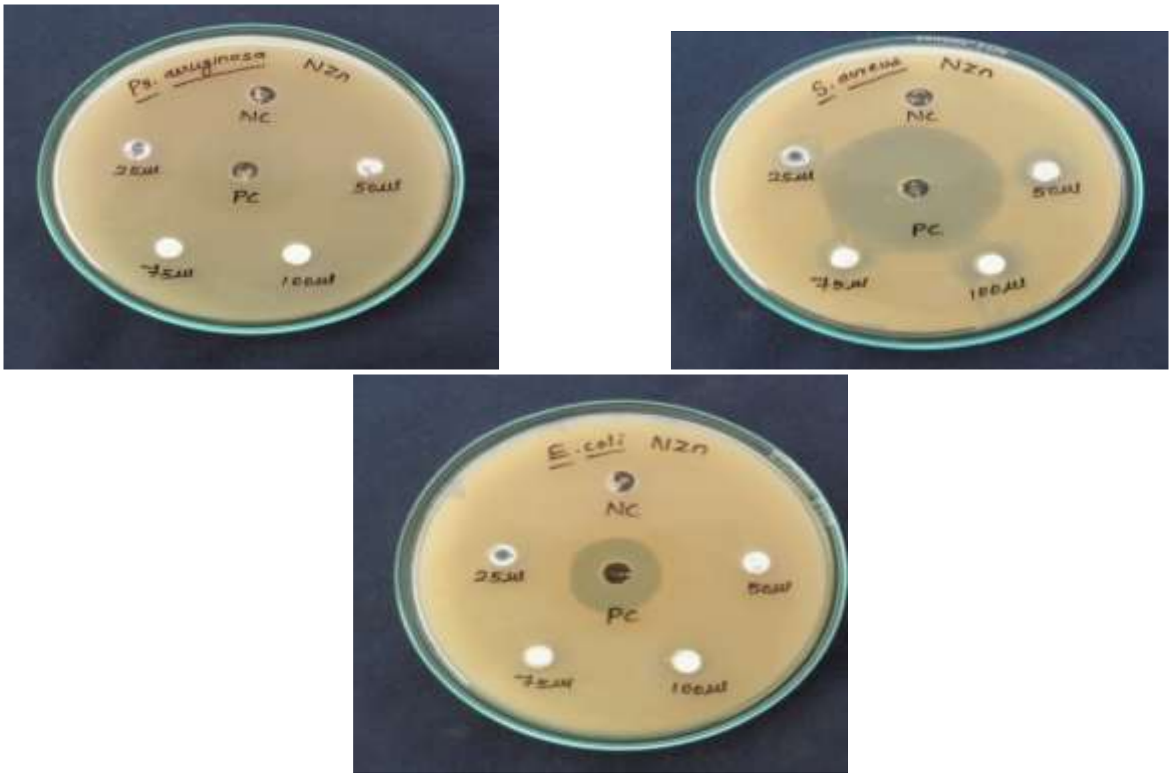

Table 4: The Zone of inhibition obtained by ZnO nanoparticles using *Morinda citrifolia* leaf extract

S.	Sample	Sample Concentration	Zone of inhibition in (mm)		
			S. aureus	***E.coli***	***Ps. aeruginosa***
1	*Morinda citrifolia*	Standard Tetracycline	17mm	12mm	10mm
2		Control	NA	NA	NA
3		25µl	5mm	NA	NA
4		50µl	6mm	NA	NA
5		75µl	7mm	5mm	NA
6		100µl	10mm	7mm	5mm

In the present work, the leaf extract obtained from *Morinda citrifolia* shows strong activity against most of the tested bacterial strains. The results were compared with standard antibiotic drugs. In this screening work, extracts of *Morinda citrifolia* were found to be not inactive against any organism, such as gram-positive, gram-negative strains were resistant to all the extracts of *Morinda citrifolia.* The results confirms the activity of leaf extract of *Morinda citrifolia* has significant antibacterial activity[25], [26] .

5. Conclusion

The ZnO nanoparticles were successfully synthesized from leaf extract of *Morinda citrifolia* which was simple, cost-effective, eco-friendly, and green approach. The leaf extract *Morinda citrifolia* can be potentially be used as an effective reducing and capping agent for biological synthesis of ZnO nanoparticles. From XRD analysis, the size of the ZnO nanoparticle was 15.77 nm. The functional groups are attached to the surface of nanoparticles were detected using FTIR spectroscopy. From UV-Visible spectroscopy, the bandgap energy of the ZnO nanoparticles was 3.31 eV. The SEM analysis demonstrated the shape of ZnO nanoparticle which was spherical shape. The EDX analysis confirmed the presence of zinc and oxide ions in synthesized nanoparticles using the leaf extract *Morinda citrifolia*. The green synthesized ZnO nanoparticles exhibited strong levels of antibacterial activity against *S. aureus* (gram positive bacteria), *E.coli* and *S. aureus* (gram negative bacteria). Green synthesized ZnO nanoparticles using the leaf extract *Morinda citrifolia* were found to protect against bacterial and fungal pathogens, suggesting that they can also be used as effective anticancer agents for commercial and biomedical applications.

Statements and Declarations

Disclosure of potential conflicts of interest

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Conflict of interest

The authors have no relevant financial or nonfinancial interests to disclose.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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